Use of Respiratory Virus POCT in ED

are we detecting current virus strains?

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'Cos 'bea 'hel are

News > Health > Flu

England flu hotspots mapped: See areas worst hit by 'mutant strain'

Flu cases are surging across England with people being urged to go back to wearing face masks if have symptoms – and below a map highlights the worst affected hospitals in the country

NEWS By **Liam McInerney** Content Editor

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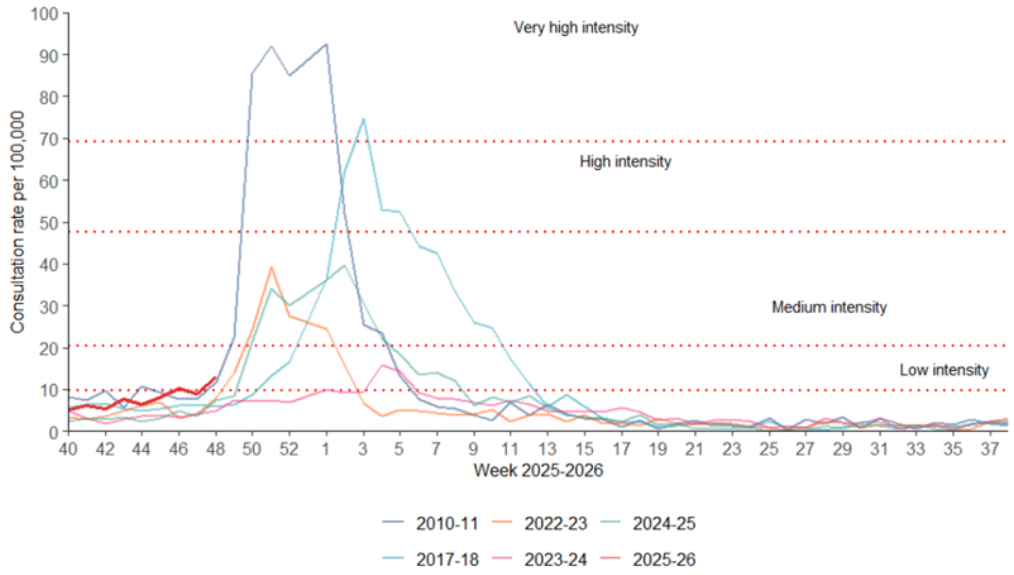
How dangerous is the 'K' strain super-flu?

Story by The Week UK • 3h • 3 min read



Data correct as of 02/12/2025

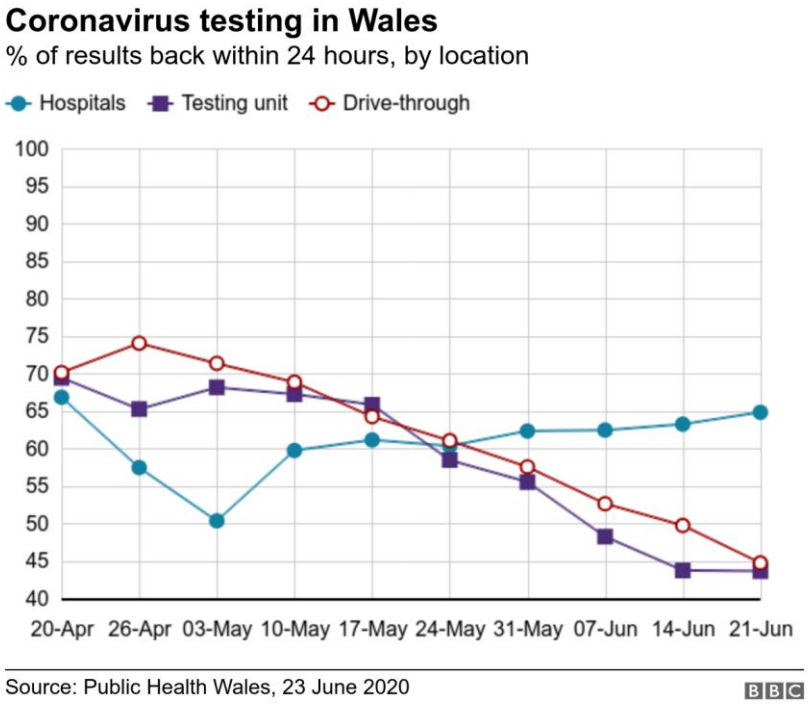
Respiratory Virus Surveillance System (Wales)

- Running for almost 40 years
  - Integrated epidemiology and laboratory services
- During 2023 recurrent funding was approved from Welsh Government to enhance surveillance in response to the COVID-19 pandemic
  - Formal establishment of the integrated surveillance team
  - Expansion of the GP sentinel network
  - Development of SARI surveillance (hospital) and expansion of community to include pharmacy
  - Establishment of the National Influenza Centre for Wales
  - Broaden remit to include molecular service development activities including emerging infection response

Virological Surveillance

Understanding the ‘normal’ patterns of viral circulation in the population and how changes that occur in that virus at the genotypic and phenotypic level might affect disease burden.
By understanding the ‘normal’, the ‘abnormal’ can be detected more rapidly.

Passive Surveillance

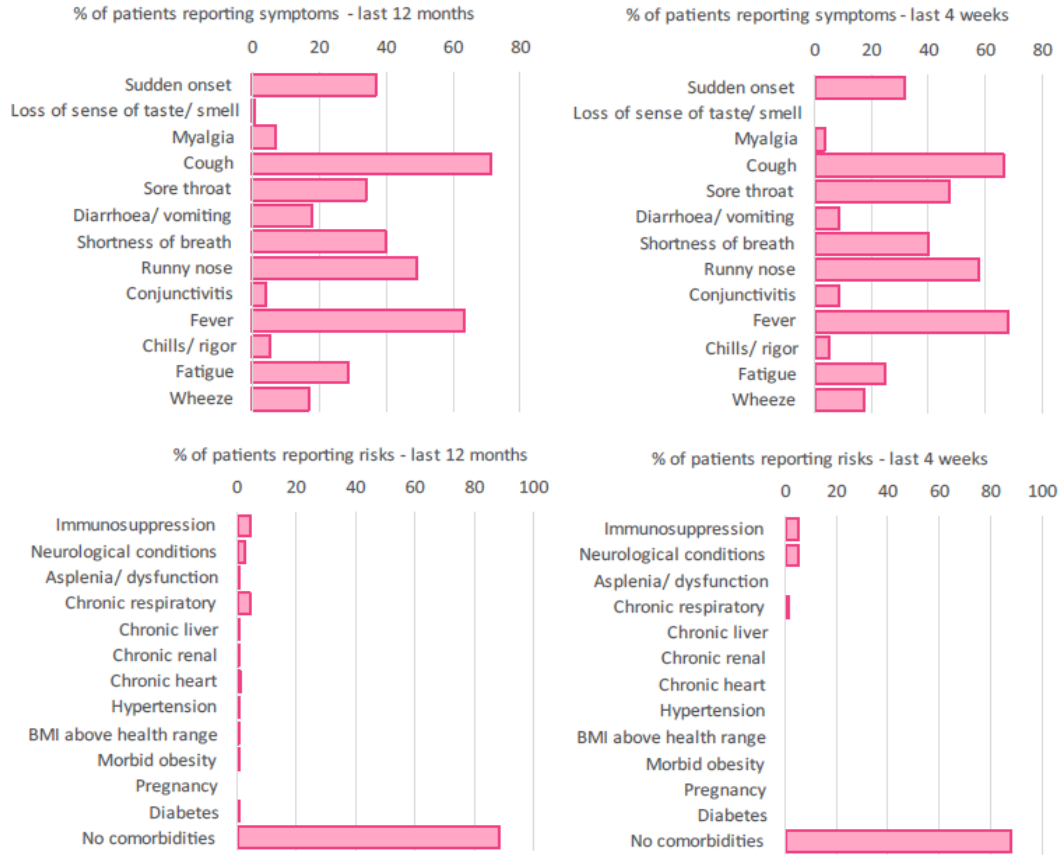


Limited information beyond numbers

Good for trend monitoring

Active Surveillance

Figure 2. Distribution of symptoms and reported comorbidities in patients accessing urgent hospital care, presenting with at least one ARI symptom and whose specimens were submitted for respiratory panel virological testing, between week 8 2024 to week 8 2025 and in the last 4 weeks.



The Welsh community Sentinel surveillance scheme
Includes one of the most comprehensive testing panels of community patients globally.

Since the emergence of SARS-CoV-2 and the following pandemic, the WHO recognised that understanding the circulation patterns and clinical symptoms associated with respiratory viruses other than influenza should be understood better.

We started this journey of expansion beyond influenza 20 years ago, with the panel almost complete 10 years go.

Virology - Respiratory inc COVID19

RESP Test Method	Luminex
Coronavirus SARS CoV 2 PCR	RNA not detected
Influenza A PCR	RNA not detected
Influenza B PCR	RNA not detected
RSV PCR	RNA not detected
Adenovirus PCR	DNA not detected
Parainfluenza PCR	RNA not detected
Rhinovirus/Enterovirus PCR	RNA Detected
Seasonal CoV PCR	RNA not detected
HMPV PCR	RNA not detected
Bocavirus PCR	DNA not detected
Chlamydophila PCR	DNA not detected
Legionella PCR	DNA not detected
Mycoplasma PCR	Result to follow

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Sequenced to understand variant circulation – early warnings of change

Sub-typed and Sequenced to understand seasonal variation– provide information for vaccine effectiveness and future composition

Now more focus on RSV due to the implementation of the vaccine programme

The seasonal respiratory viruses
Contributing to significant morbidity in the general population, immunity is not complete and severity increases in at risk populations. No vaccines, few treatment choices, but monitoring through sentinel surveillance helps build a body of evidence for epidemiology, impact and future targets for vaccines and treatments

Atypical bacterial infections, can be treated. Not classically thought to be significant drivers of mild community illness

Expansion of the sentinel testing repertoire was largely in place in 2014 and completed in 2018 with the addition of the seasonal coronaviruses
SARS-CoV-2 implemented in 2021 – but numbers were small

What is multiplex testing?

- A means by which a single clinical syndrome is investigated simultaneously for multiple likely causes using diagnostic tools
- More often laboratory based
- Can be performed by different methods
  - Infection
    - Multiple sample types using a combination of culture, serology and/or molecular
    - Purely molecular, can be achieved from a single sample

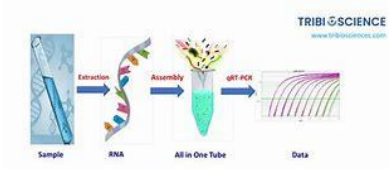
Syndromic Testing

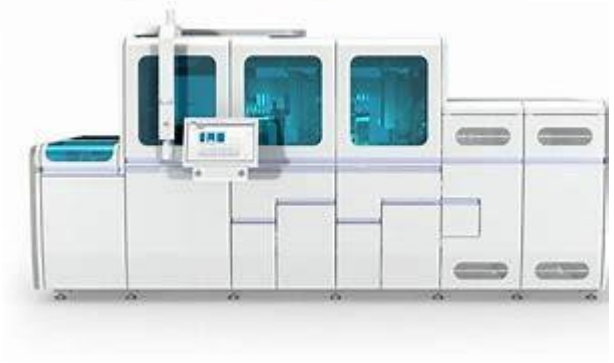
PCR Panels

- Panels are increasing in complexity from 2 pathogen targets to 20 and above in one test.
  - Sexual Health
  - Respiratory
  - Gastrointestinal infections
  - Meningitis/encephalitis
- Rapid turnaround time associated with the sample to answer platforms
  - 15 to 60 minutes
- Increased use closer to the patient
  - Primary care
  - ED
  - Pharmacy

Multiplex PCR platforms

A plethora of choice



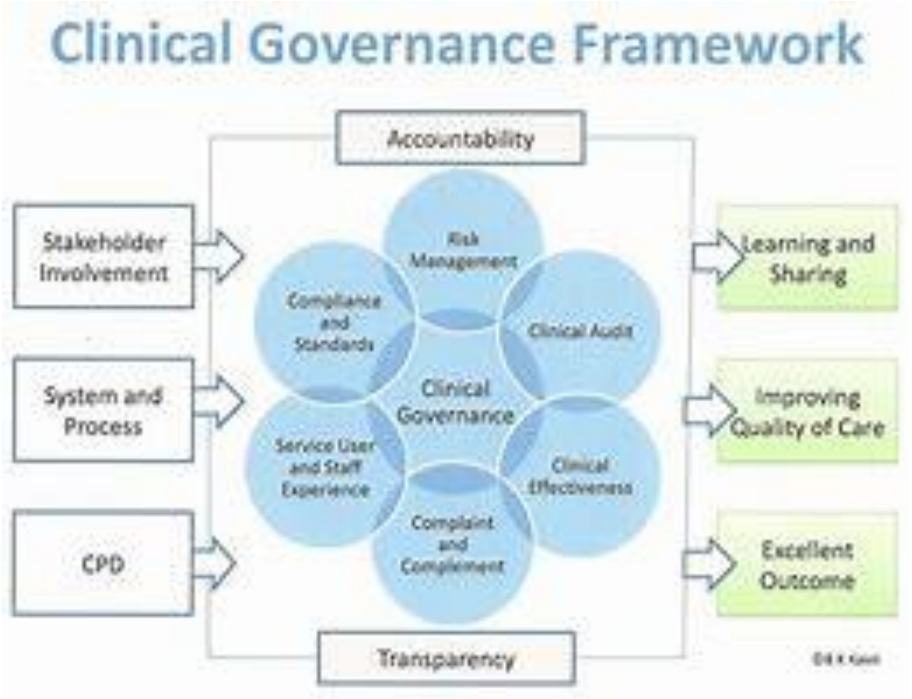


Classical Molecular

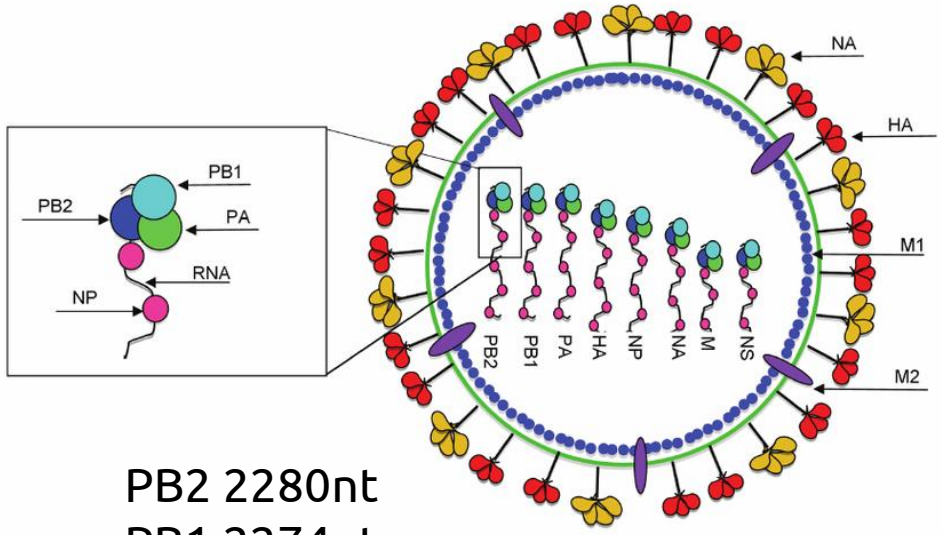
Sample to Answer

Challenges
Pre-pandemic

- Cost
- Validation and verification
- Quality assurance – EQA/IQA
- Proficiency
- Control material
- Result collation and reporting
- Sample referral
- Dual/triple/quadruple infections

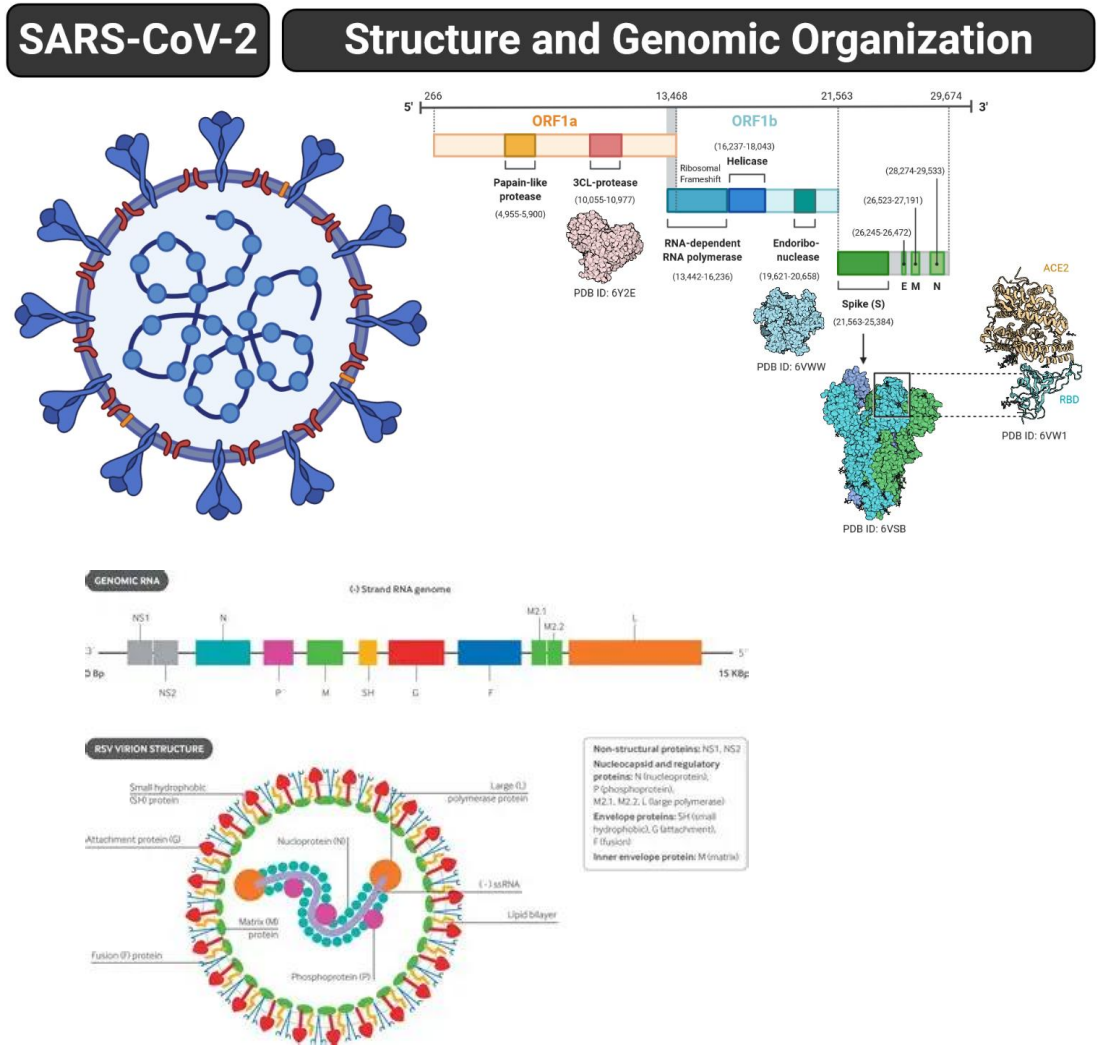


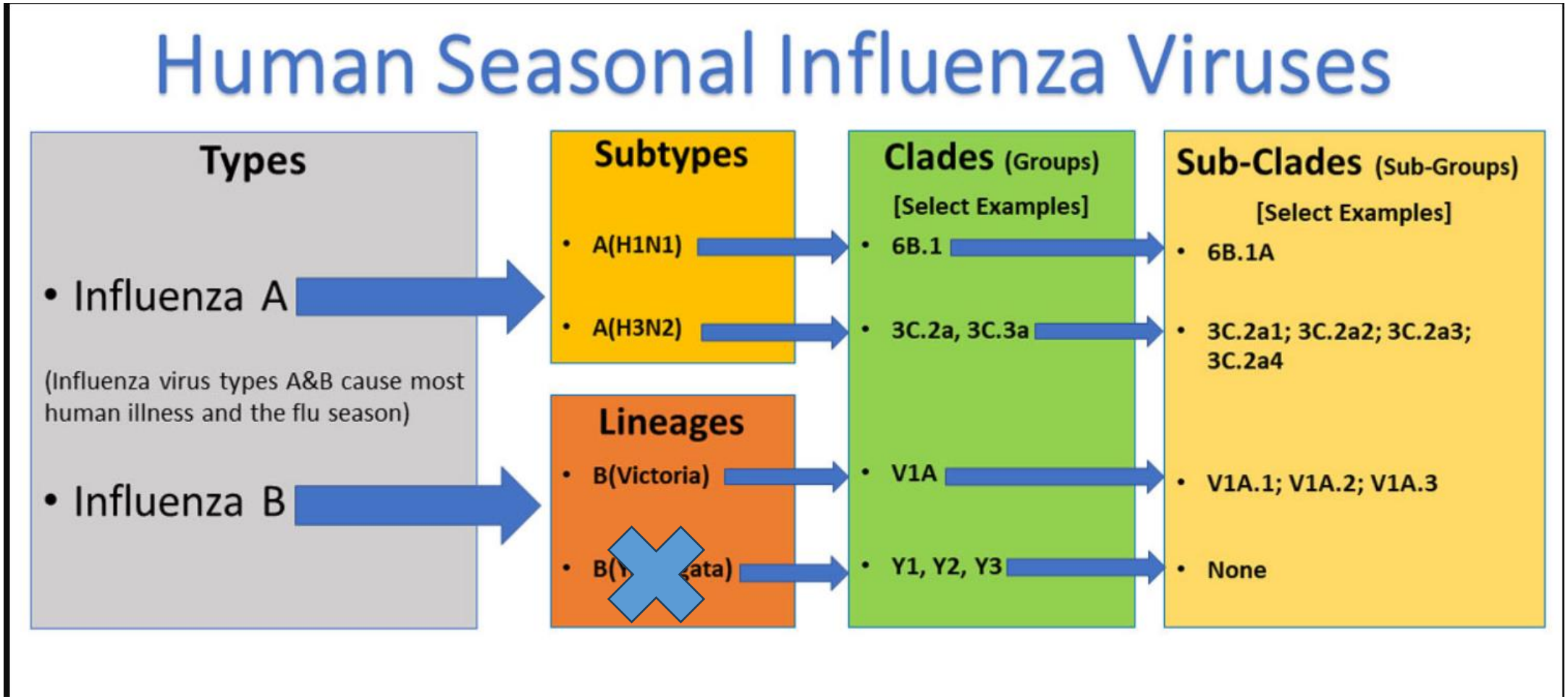
Genome of Influenza A (and B)



PB2 2280nt
PB1 2274nt
PA 2151nt
HA 1701nt
NA 1410nt
NP 1479nt
MP 982nt
NS 838nt

The antibody response to the influenza A virus typically targets the surface glycoproteins HA and NA, while the T-cell mediated response typically targets the relatively conserved internal proteins including NP, M1, and PB1.





PCR
Internal genes e.g Matrix, PB2

PCR
Haemagglutinin
Neuraminidase

Genomics

Antigenic Drift v Genetic Drift

- Antigenic/genetic drift arises because of the inherent error prone replication of viral RNA leading to point mutations in genes coding HA and NA.
- If the drifted strain has mutations in two or more antigenic sites of HA epidemics may occur as more people become susceptible.
- Genetic drift occurs at any point in the genome and usually relates to nucleotide changes that can be synonymous or non-synonymous.
- The rate of these changes across the genome for single stranded RNA viruses such as influenza are many times greater than those for DNA viruses
- Most changes are deleterious to the virus, however some are advantageous, increasing for example host range
- Genetic drift can and does cause problems for diagnostics, even if antigenicity isn't affected – goes beyond influenza

Viral Mutation – Impact on Testing

- All viruses mutate over time – rates vary by nucleic acid type and immune pressure
- Coronaviruses have some level of proof-reading and production of sub-genomic RNA is used as part of the replication strategy; so changes are typically slower than those of other RNA viruses
- Most mutations are synonymous, that is the downstream protein isn’t affected
- However, a single nucleotide change can affect assay sensitivity
  - Monitoring for these changes are challenging in a commercial system

Molecular test principles

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for Wuhan virus will be provided shortly.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay
Additional confirmatory assay: N gene assay

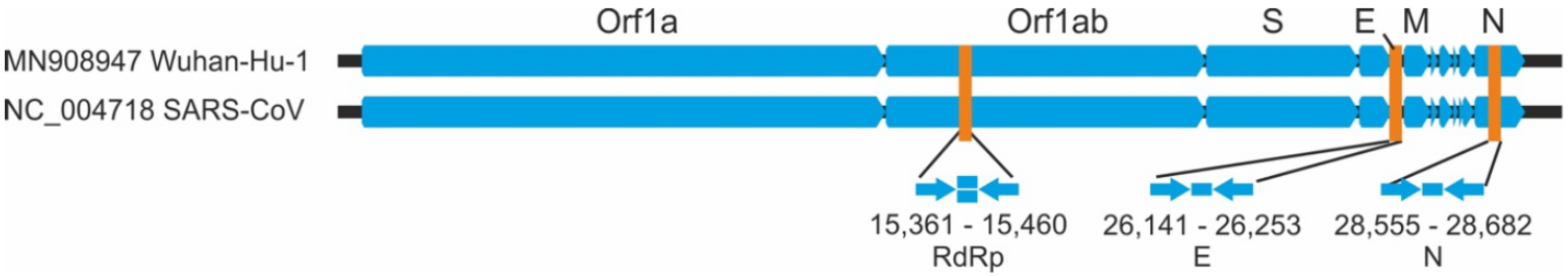
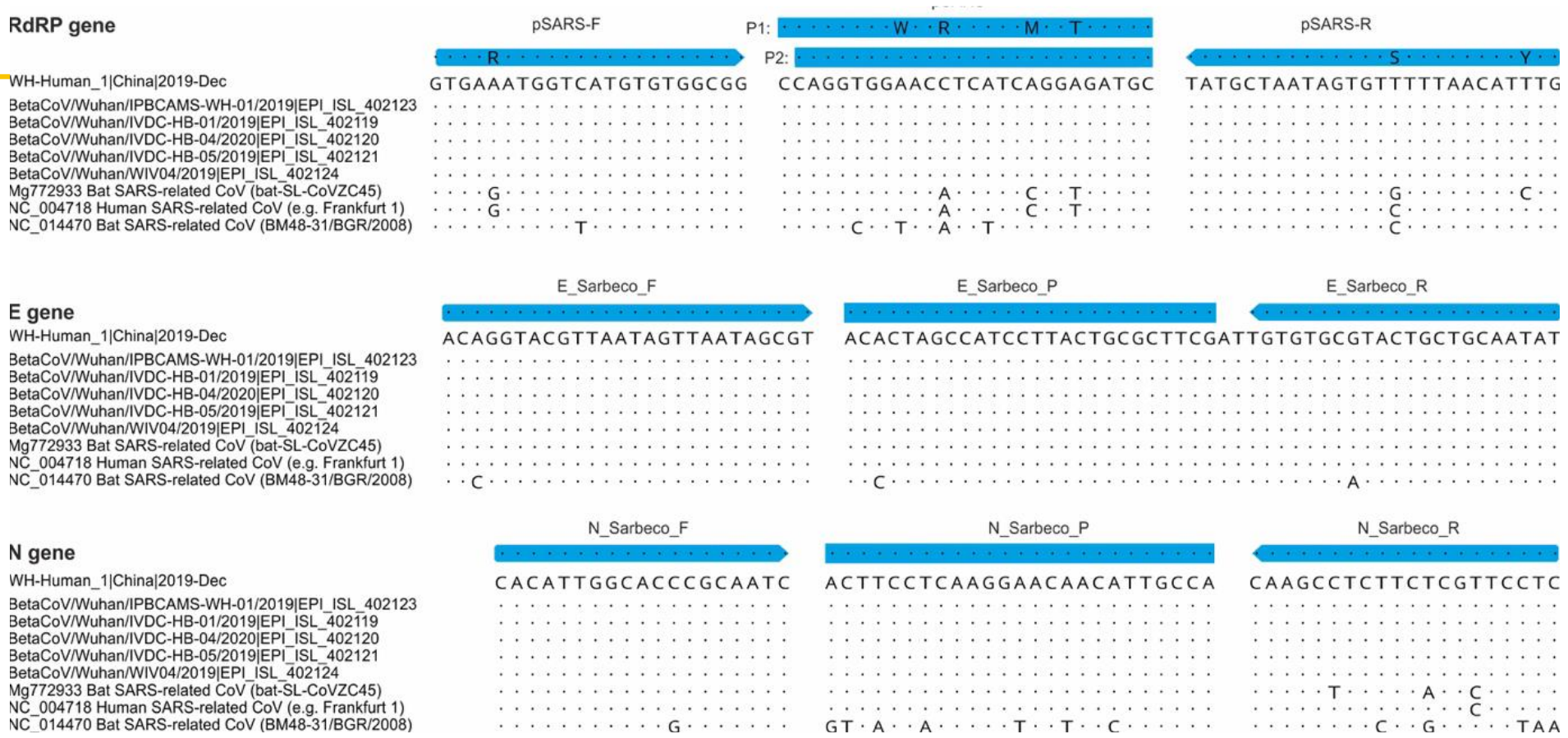


Figure 1 relative positions of amplicon targets on SARS-CoV ad Wuhan-CoV genome. N: nucleocapsid; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

Mutations in primer and probe regions



Example results

Sample 4

Gene	SeeGene original	SeeGene frozen thawed	Rhyl Starlet	Roche	Perkin Elmer	Carmarthen Cepheid	Aries Rhyl
E	27.57	27.48	28.57	27		26.6	
RdRp	29.28	29.2	29.5	27	25.9		27.8
N	30.05	30.3	31.14		26.3	28.2	29.2

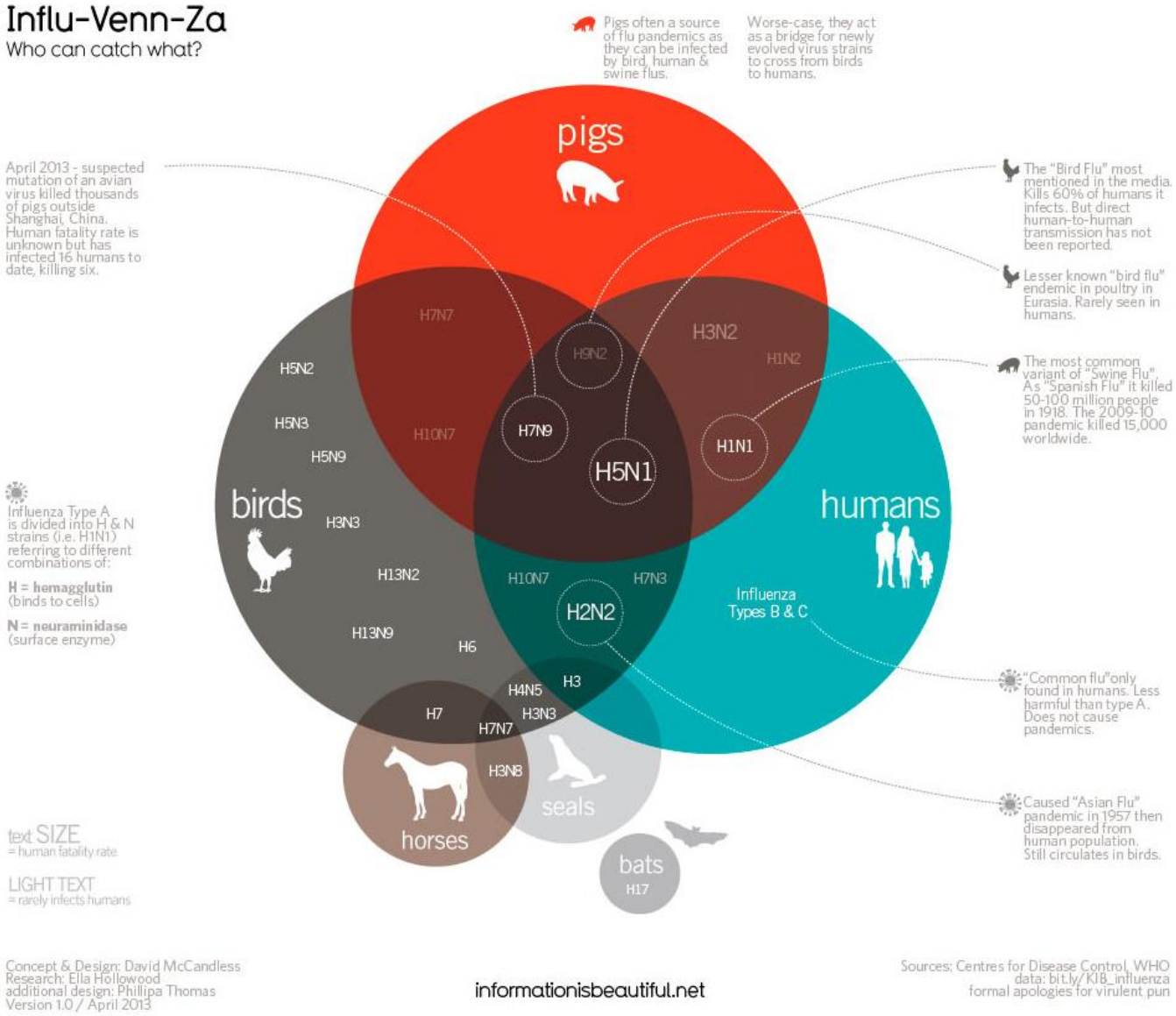
Detection comparable for SeeGene, Roche, Luminex NxTag, Perkin Elmer, Cepheid, Hologic, ePlex – slightly less sensitivity observed for ARIES

Lower level of detection

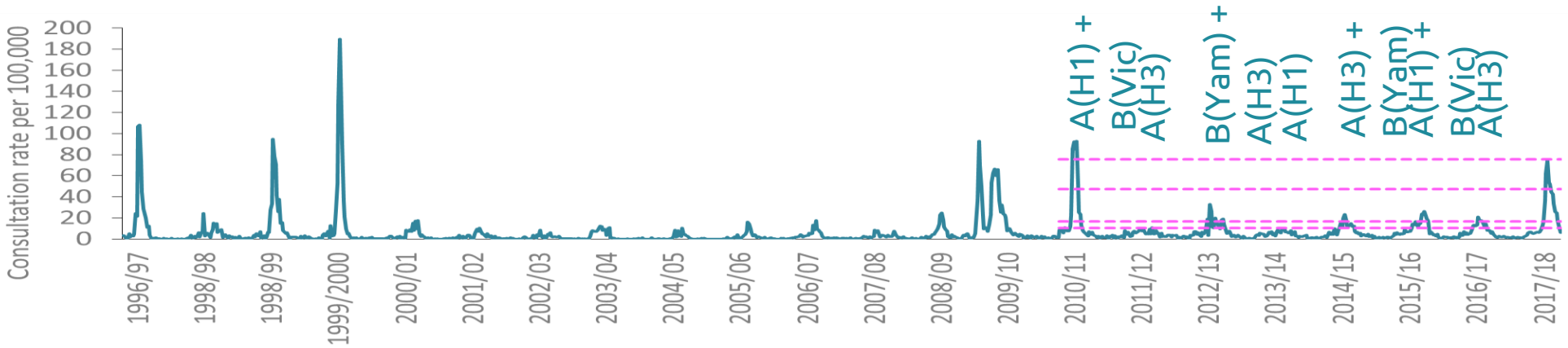
Lots of variation

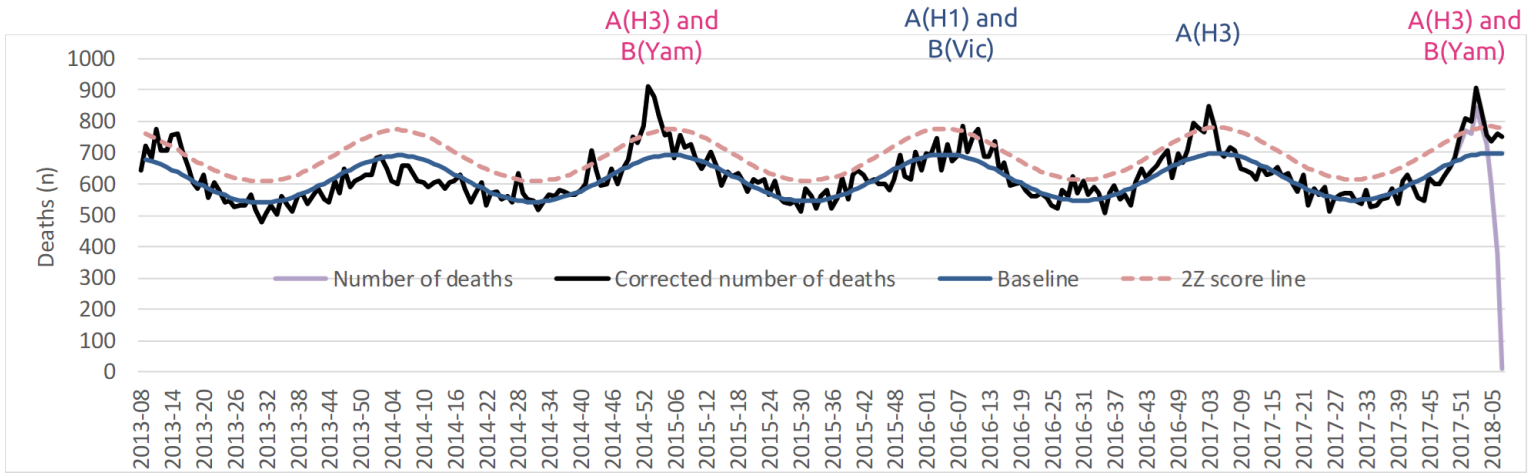
Gene	Seegene Cardiff	Bangor Nimbus	Newport Cepheid	Bangor Cepheid	Swansea Aries	Luminex NxTag	Roche
E	35	33	36	34		N	
RdRp	36	34			N	N	N
N	33	33	N	N	29	N	N

Not detected results also recorded for Hologic and Perkin Elmer



131 different combinations of HA and NA have been discovered in nature from a possible 198





Influenza seasons are referred to by the sub-type that circulated.

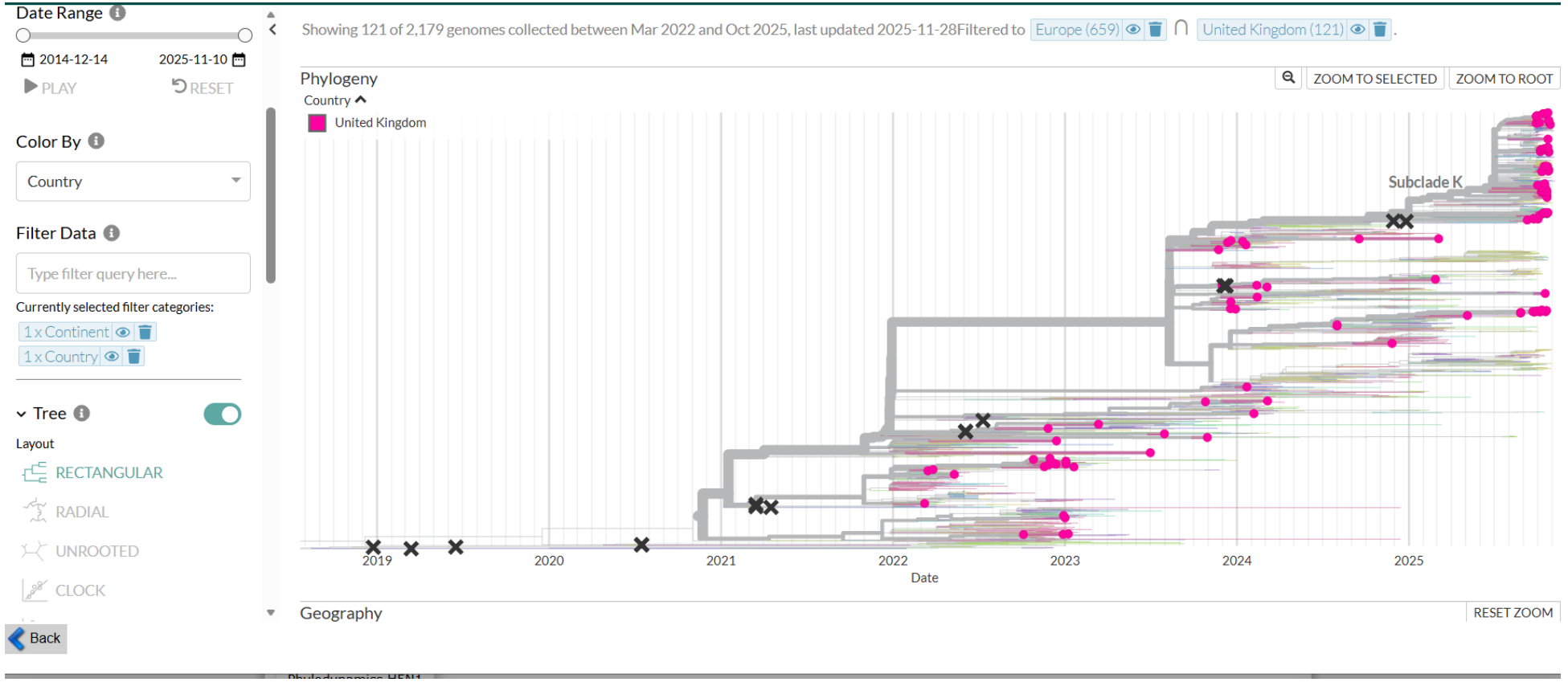
H3N2 seasons are notable for higher rates in the elderly with carehome outbreaks and increased mortality

H1N1pdm09 is typically an infection of younger adults and children

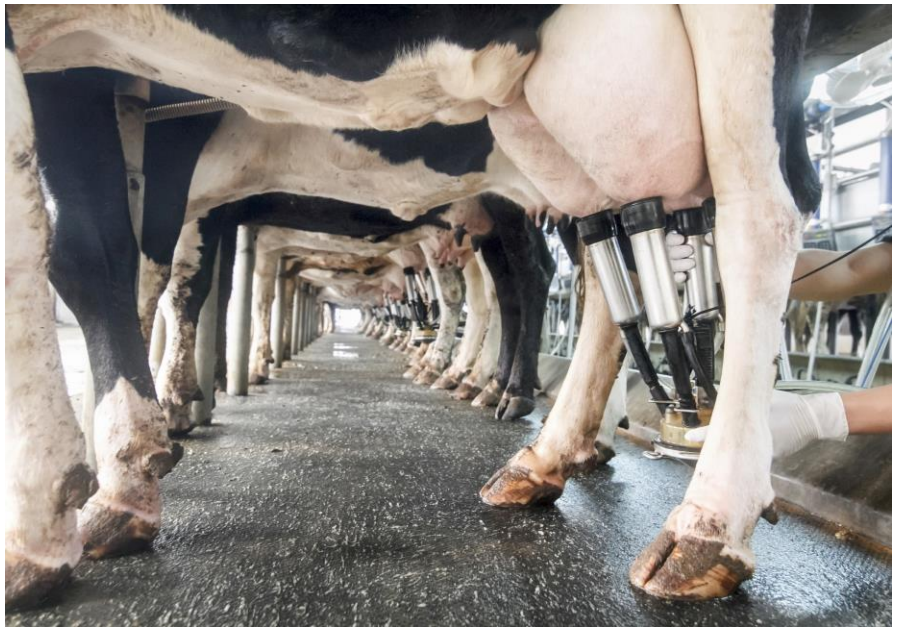
Influenza B is an underestimated cause of morbidity in all age groups, but again focussed on young children

Understanding sub-type early in the season allows for alerts, not only about 'if the vaccine will work' but also potential impact in secondary and tertiary care

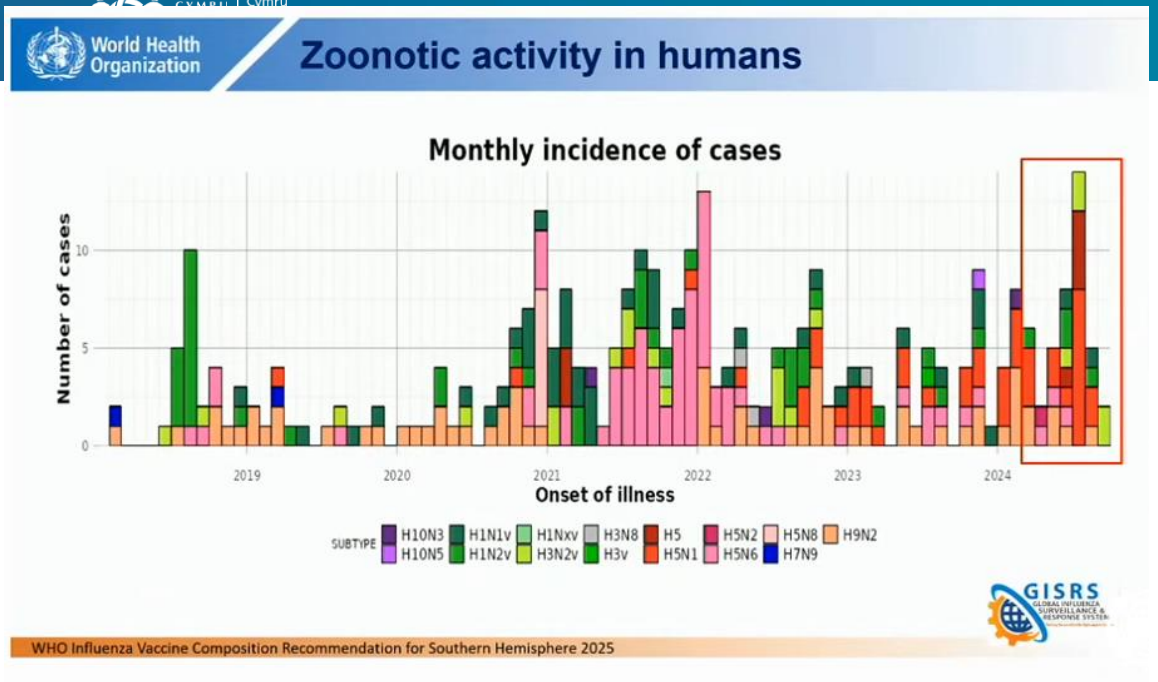
Influenza H3N2 viruses over time - UK

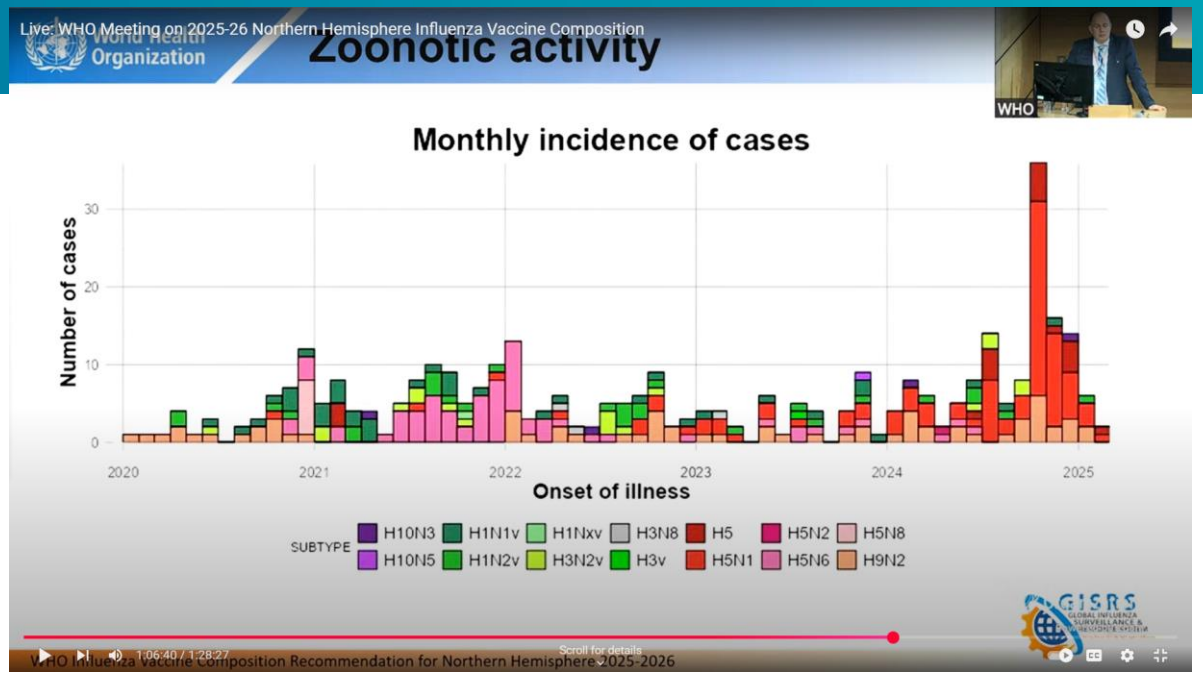


Avian Influenza in Cows – Bovine influenza A



- The influenza A virus causing the bovine infections belongs to the clade 2.3.4.4b H5N1 virus that has caused a panzootic (pandemic in birds)
- The virus reassorted with a low pathogenic bird influenza in the US clade 3.13 so differs from that in Europe
- Infection of livestock was likely through a single introduction from a bird, with transmission ongoing via mechanical means
- The virus has adapted to the cells of the bovine mammary tissue but retains avian properties – including cell receptor preference
- Infections in felids have been fatal (direct ingestion)
- In humans, infections have been mild and include conjunctivitis
- Recently H5N1 subclade D1.1 has also spilled over into cattle, this virus caused more severe forms of human disease





Human infections with non-human influenza from avian and swine sources occurs frequently

Most often infections arise from direct contact with infected animals

Avian influenza in particular causes high rates of environmental contamination – leading to increased risk of spillover events during epidemics

Detection of recent sporadic cases have been detected through sentinel schemes where influenza A samples are unable to be typed using seasonal sub-typing assays, this leads to further investigation and deployment of animal influenza specific assays – e.g. H5, H7, H9

Influenza Reassortment in Man

RAPID COMMUNICATIONS

Case of seasonal reassortant A(H1N2) influenza virus infection, the Netherlands, March 2018

Adam Meijer[1,6], Corien M Swaan[1], Martin Voerknecht[2], Edin Jusic[1], Sharon van den Brink[1], Lisa A Wijsman[1], Bettie CG Voordouw[1,6], Gé A Donker[3], Jacqueline Sleven[4], Wendelien W Dorigo-Zetsma[5], Sanela Svraka[5], Michiel van Boven[1], Manon R Haverkate[1], Aura Timen[1], Jaap T van Dissel[1], Marion PG Koopmans[6], Theo M Bestebroer[6], Ron AM Fouchier[6]

1. Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands
2. General practitioner participating in the Primary Care Database sentinel surveillance coordinated by NIVEL Netherlands institute for health services research, Utrecht, the Netherlands
3. Coordinator NIVEL Primary Care Database sentinel surveillance, NIVEL Netherlands institute for health services research, Utrecht, the Netherlands
4. Municipal Health Services 'Gooi en Vechtstreek', Bussum, the Netherlands
5. Central Bacteriology and Serology Laboratory, Tergooi Hospitals, Hilversum, the Netherlands
6. Department Viroscience, Erasmus University Medical Center, Rotterdam, the Netherlands

Correspondence: Adam Meijer (Adam.Meijer@rivm.nl)

Reassortment can also be intra-subtypic (gene swapping between viral sub-clade)
Reassortment between human and non-human viruses can lead to the emergence of a pandemic strain

Detection of Spillover/emerging influenza in the Routine Laboratory

- In a good generic influenza A assay human and non-human influenza should report the same/similar results
- Unless history consistent with exposure to infected animals is given, no reason to look further
- Subtyping capability flags a problem – especially in samples with good ct value

		Platform A generic		Platform B generic		Platform C generic		Platform D generic and type		Platform E generic and type			CDC Influenza A		In-house H1/H3		In-house H5a		In-house H5b 2.3.4.4b	
	Dilution	Result	Ct Value	Result	Ct Value	Result	Ct Value	Generic A (T35)	Typing (T 45)	Flu A pan1	Flu A pan 2	Flu A H1 2009	Result	Ct Value	Result	Ct Value	Result	Ct Value	Result	Ct Value
Seasonal influenza H1N1pdm09	10-2	Positive	28	Positive	24/26	positive	25	370	442	13.9	20.1	22	Pos	26	Pos	36	neg			
	10-3	Positive	32	Positive	27/30	positive	28	367	442	16.7	23	25	Pos	30	Pos	39	neg			
	10-4	Positive	35	failed		positive	32	372	100	20.6	26.9	30	Pos	34	Neg		neg			
	10-5	Neg		Positive	35/37	positive	34	58	32	24.4	Neg	no cp value	Pos	36	neg		neg			
	10-6	Neg		Neg		Negative		-1	3	Neg	Neg	Neg	Neg		neg		neg			
	10-7	Neg		Neg		Negative		0	0	Neg	Neg	Neg	Neg		neg		neg			
H5N1 (Bovine)	10-2	Positive	27	Positive	23/26	positive	26	437	-1	17.7	27	Neg	Pos	28	neg		Pos	32	Pos	27
	10-3	Positive	31	Positive	27/29	positive	34	276	-1	19.7	30	Neg	Pos	32	neg		Pos	35	Pos	30
	10-4	Positive	33	Positive	31/33	positive	33	93	-2	22.8	30	Neg	Pos	35	neg		Pos	39	Pos	33
	10-5	Positive	37	Positive	35/37	positive	35	0	0	25.6	Neg	Neg	Pos	37	neg		Pos	>40	Pos	37
	10-6	Neg		Neg		Negative		0	0	Neg	Neg	Neg	Neg		neg		neg		Neg	
	10-7	Neg		Neg		Negative		0	0	Neg	Neg	Neg	Neg		neg		neg		Neg	

Failure of a generic influenza assay to reliably detect seasonal influenza A H1N1pdm09 - 2022/23

- The issue was first detected by the Belfast laboratory, Cardiff and Glasgow were contacted to see if we were seeing the same problem with H1N1pdm09 detection on a commercial platform
- The problem was only detected when a sample that would normally be reported as low level was sub-typed. The sub-typing PCR suggested a ct value that was lower than that reported by the generic assay.
- This finding was confirmed in Cardiff and Glasgow – reported to MHRA and UKHSA
- Current H1N1pdm09 viruses are causing detection dropouts and reduction in sensitivity in a number of platforms
- Also seen in previous years for H3N2 and influenza B

Episode number	sample date	Original testing method	Original result	Flu A in-house Ct value	Roche ct
7021929272	23/08/2022	biofire	H1N1 2009	27.69	ct 35 on Roche
7022169263	04/09/2022	biofire	H1N1 2009	22.09	ct 35 on Roche
7022324998	10/09/2022	biofire	H1N1 2009	24.7	ct 36 on Roche
7022358840	12/09/2022	biofire	H1N1 2009	20.74	ct 30 on Roche
7022690480	26/09/2022	biofire	H1N1 2009	20.98	ct 39 on Roche
7022771170	29/09/2022	biofire	H1N1 2009	24.71	negative on Roche
1203208239	29/09/2022	Seegene	influenza a (ct 29)	24.48	ct 38 on Roche
7022761073	30/09/2022	Seegene	Influenza A (ct 27)	24.71	ct 37 on Roche
7022946079	09/10/2022	Seegene	influenza a (ct 27)	24.65	negative on Roche
7022994700	09/10/2022	biofire	H1N1 2009	24.65	negative on Roche
7022998705	09/10/2022	biofire	H1N1 2009	23.51	negative on Roche
7022995492	10/10/2022	Seegene	influenza a (ct 19)	18.82	ct 36 on Roche
1203537109	10/10/2022	biofire	H1N1 2009	25.85	ct 37 on Roche
7023058703	11/10/2022	seegene	influenza a (ct 26)	23.76	ct 37 on Roche
7023093172	12/10/2022	biofire	H1N1 2009	24.92	negative on Roche
7023009519	12/10/2022	Seegene	influenza a (ct 22)	22.88	ct 32 on Roche
7023070218	13/10/2022	seegene	influenza a (ct 29)	24.77	negative on Roche
7023075993	13/10/2022	seegene	influenza a (ct 25)	24.7	negative on Roche
7023263752	20/10/2022	biofire	H1N1 2009	22.75	ct 35 on Roche
7023292716	20/10/2022	Seegene	influenza a (ct 30)	27.08	not affected
7023311535	21/10/2022	Biofire	H1N1 2009	20.85	ct 32 on Roche
7023312497	21/10/2022	biofire	h1N1 2009	29.7	negative on Roche
7023187610	21/10/2022	Seegene	influenza a (27)	25.88	ct 35 on Roche
1203537552	23/10/2022	Biofire	H1N1 2009	24.15	ct 37 on Roche
7023330251	23/10/2022	biofire	H1N1 2009	28.47	not affected
7023360134	24/10/2022	biofire	H1N1 2009	27.88	negative on Roche
7023382940	25/10/2022	seegene	influenza A (30)	25.7	ct 37 on Roche
7023392236	25/10/2022	biofire	H1N1 2009	24.65	ct 35 on Roche
7023390363	25/10/2022	biofire	H1N1 2009	19.97	ct 37 on Roche
7023457331	27/10/2022	biofire	H1N1 2009	25.27	ct 37 on Roche
7023465454	28/10/2022	biofire	H1N1 2009	27.09	negative on Roche
1003310531	no date	Seegene	influenza a (ct 27)	27.29	ct 37 on Roche

Summary

- POCT is being used more widely for respiratory viruses in ED
- The viruses being targeted are prone to mutation that can affect diagnostics
- Missing a late infection due to poor sensitivity may have limited impact
- Missing an infection due to a change in the virus may have mor significant implications
- EQA and IQA can go some way to diagnostic assurance
- Please report and refer to public health positive samples to allow follow-up and virological surveillance.

Acknowledgements

- The staff from the NIC, WSVC, the Welsh lab network
- The combined surveillance team
- Welsh Government

Thank you for listening.
Any questions?

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