

Increased efficiency to meet winter demands for rapid detection of respiratory viruses

Gemma Clark, PhD
Clinical Scientist
Nottingham University Hospitals

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Nottingham University Hospitals



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Winter has been...

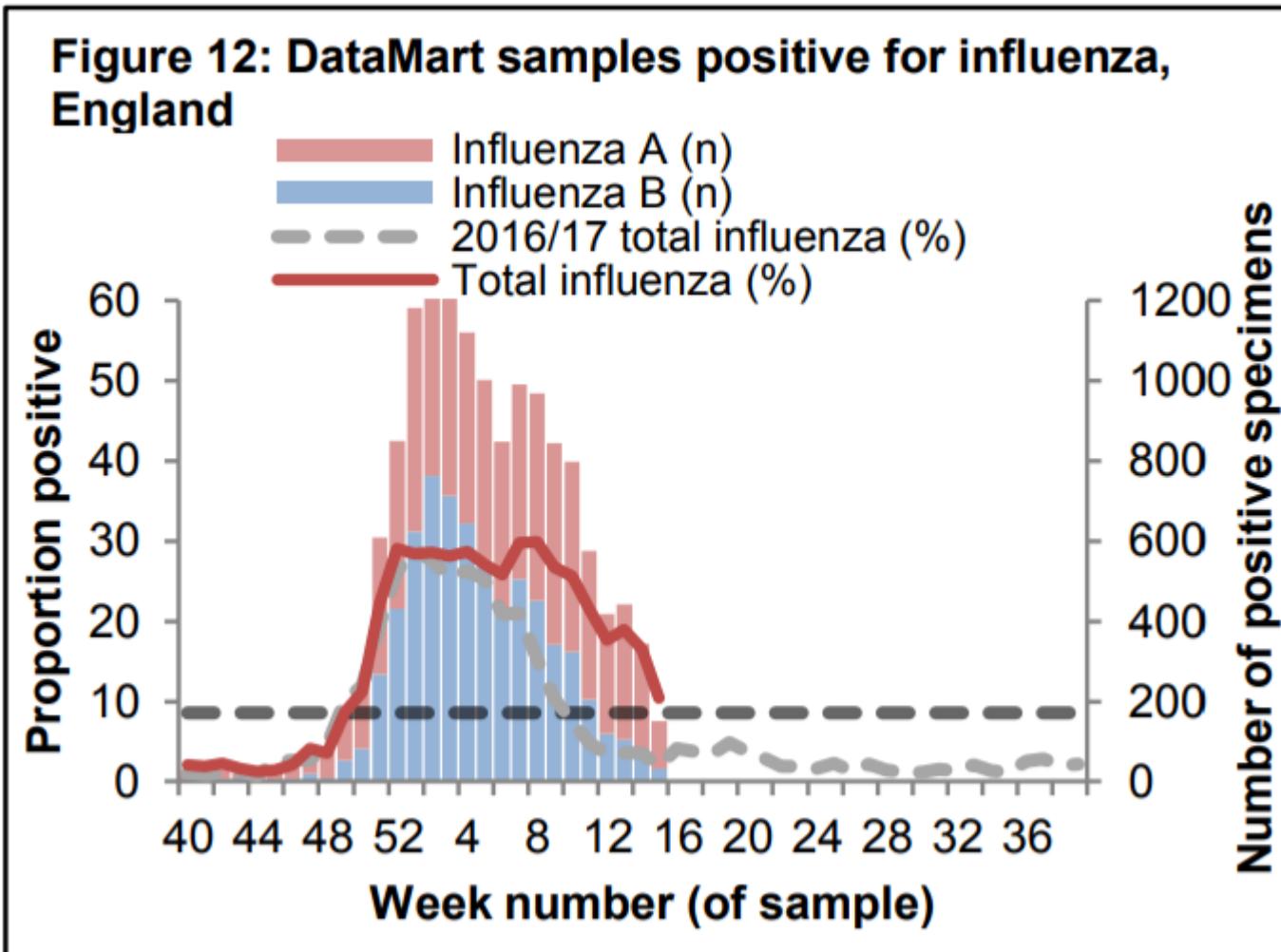
Our hospitals are very busy

Please **do not** attend the Emergency Department unless a **real emergency** and **consider alternative services**.

If you are due to come in for an operation or appointment **please attend as normal**. You will be contacted directly if it has been cancelled.

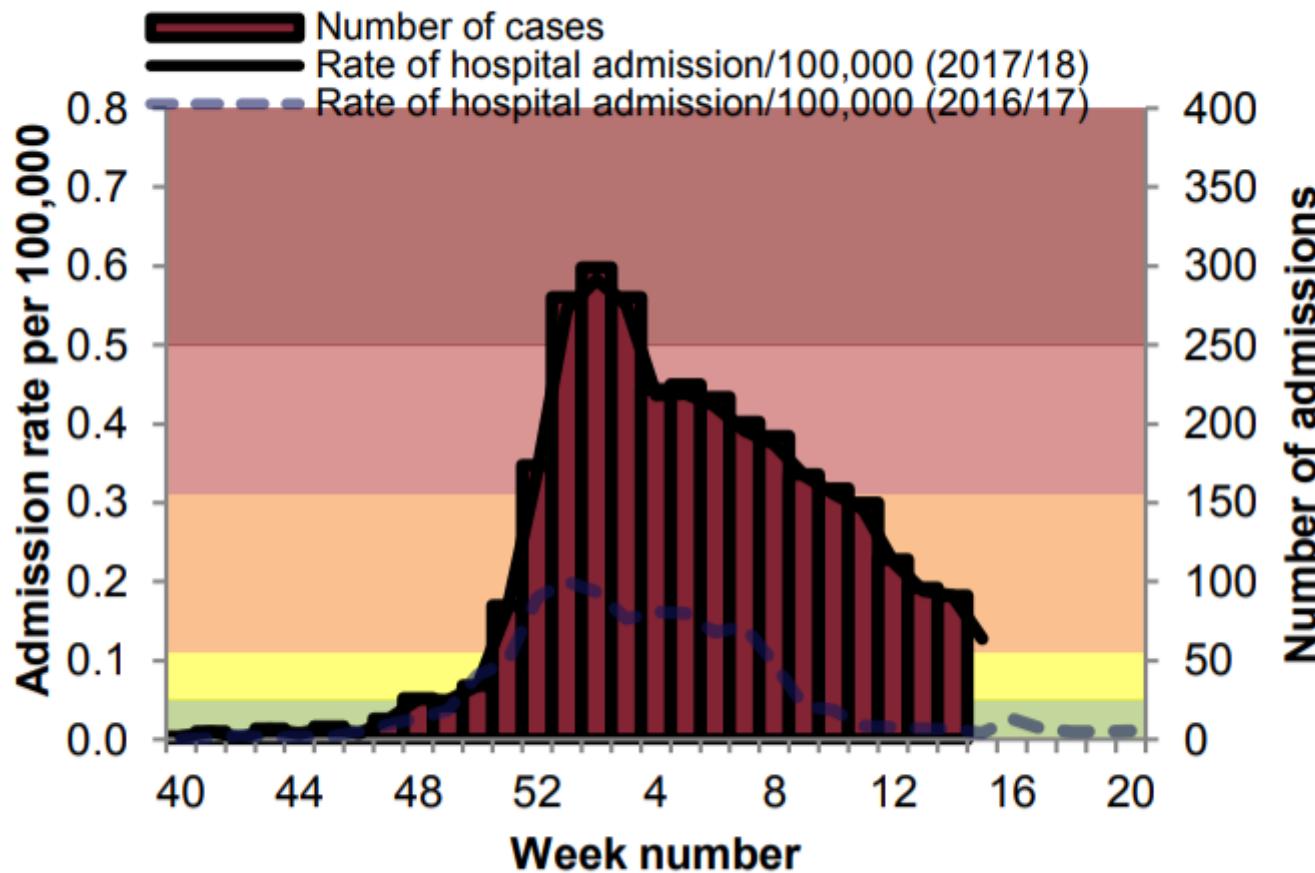
Winter flow 2018

PHE Weekly National Influenza Report



PHE Weekly National Influenza Report

Figure 6: Weekly ICU/HDUinfluenza admission rate per 100,000 trust catchment population , England, since week 40 2017



Baseline threshold

Low

Medium

High

Very high

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Source: PHE Weekly National Influenza Report, week 16

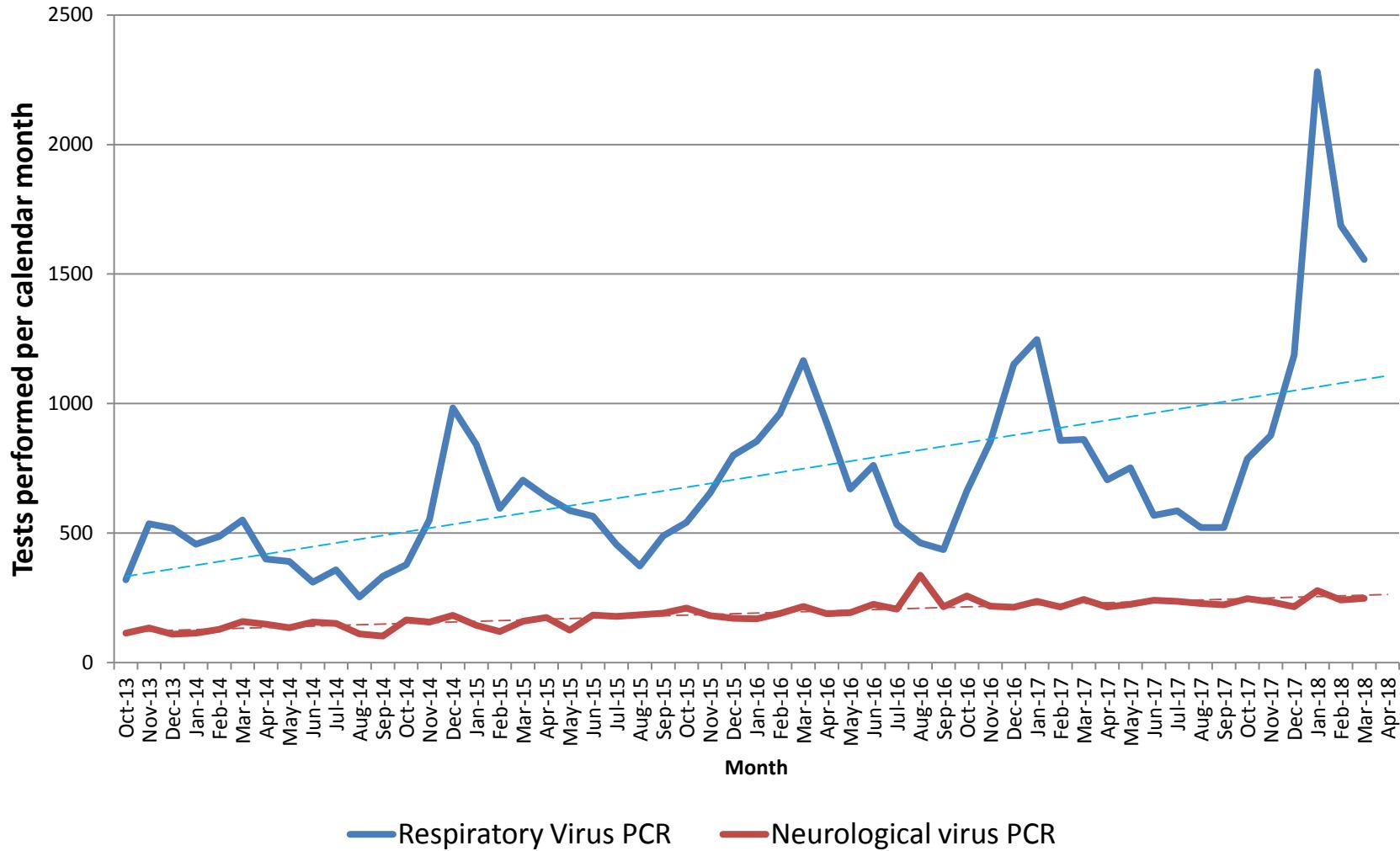
Seasonal influenza vaccine

- WHO recommendations for 2017-18 Northern hemisphere trivalent vaccine:
 - an A/Michigan/45/2015 (H1N1)pdm09-like virus
 - an A/Hong Kong/4801/2014 (H3N2)-like virus
 - a B/Brisbane/60/2008-like virus

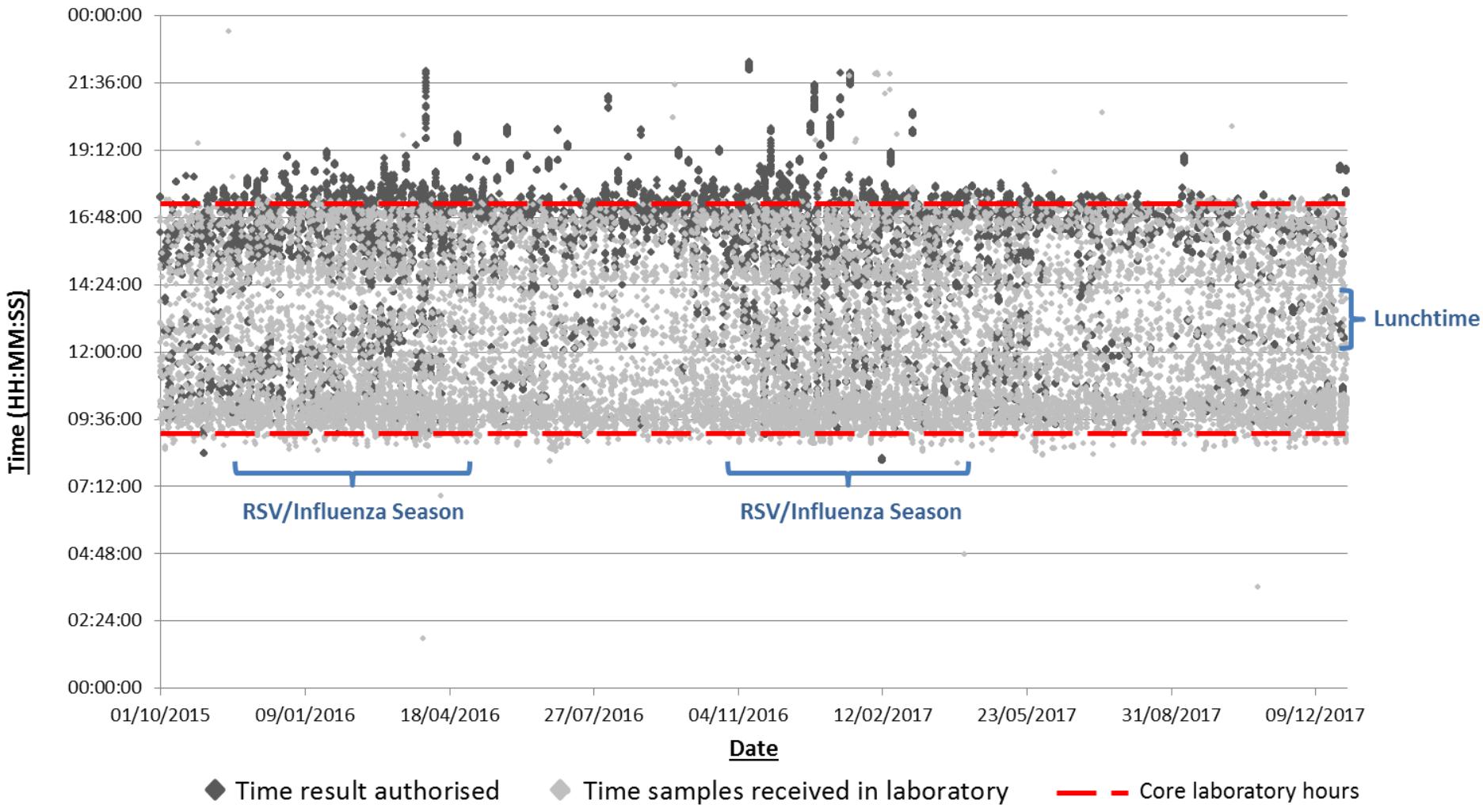
Table 3: Viruses characterised by PHE Reference Laboratory, 2017/18

Virus	No. viruses characterised			
	Genetic and antigenic	Genetic only	Antigenic only	Total
A(H1N1)pdm09	82	75	92	249
A(H3N2)	1	530	0	531
B/Yamagata-lineage	188	409	287	884
B/Victoria-lineage	7	0	0	7

Molecular diagnostics workload analysis

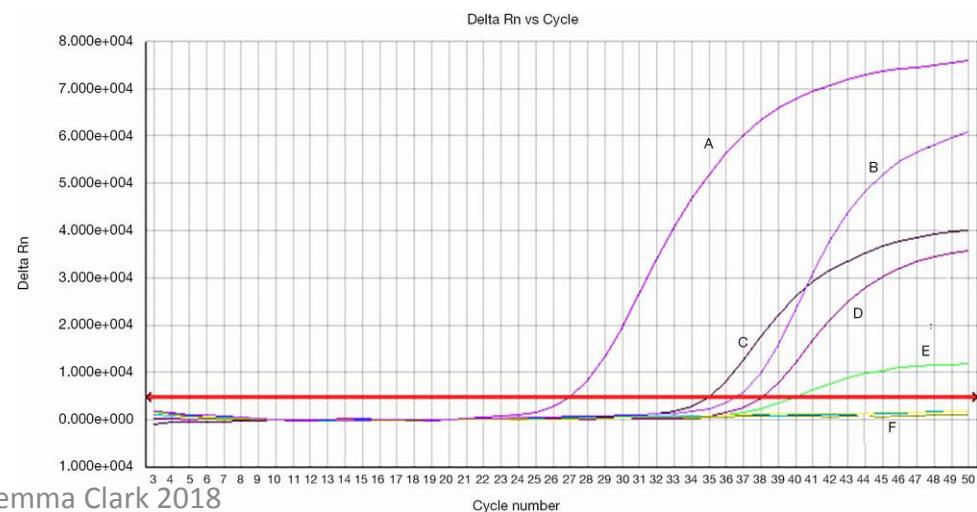


Sample receipt and authorisation analysis



Respiratory viruses PCR c.2015

- In-house assay
 - 96-well plate-based assay
 - 5 mastermixes
 - Manual PCR plate set up
 - Manual results interpretation



Respiratory viruses PCR c.2015

- Staffing challenges
 - Loss of experienced staff and increase in workload
 - ↑ mastermix QC failures
 - ↑ Positive control QC failures
- Performance concerns
 - EQA failures (Rhino/Enterovirus, MPV)
 - Genetic mutations?
 - Primer sensitivity/specificity

The next generation?

- Workflow analysis
 - messy!
- Shopping list:
 - Simplified workflow
 - Reduced complexity
 - Quality performance

High-Plex™ 384 SYSTEM



High-Plex analyser

STEP 1 PCR

15 PCR cycles

Multiplex

16 primer pairs

~120 minutes



High-Plex processor

STEP 2 PCR

30 PCR cycles

Singleplex

Melt curve analysis

~65 minutes



PC

Results

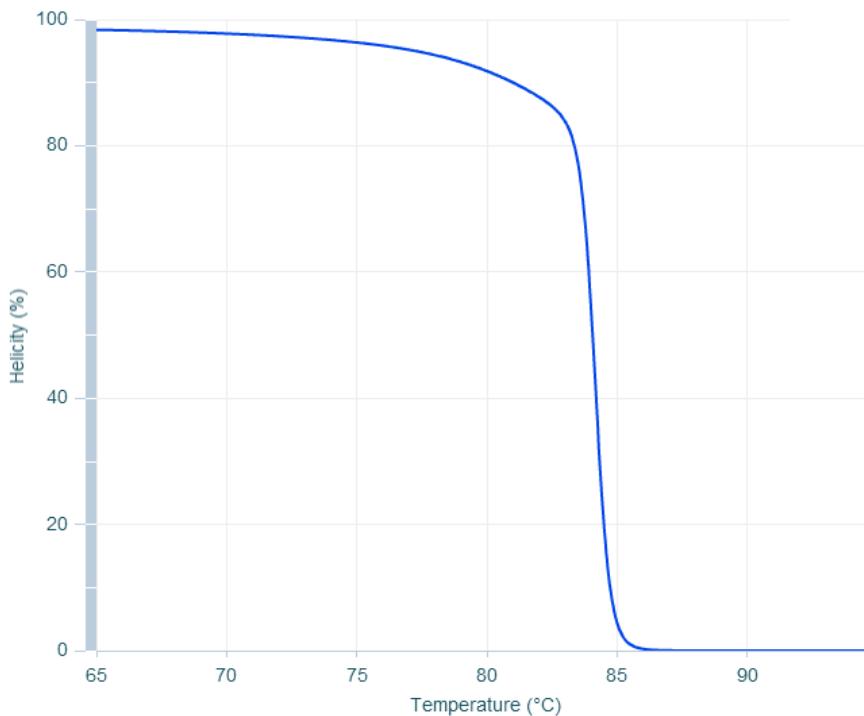
Semi-automated
analysis of real-time
PCR results

Melt curve analysis

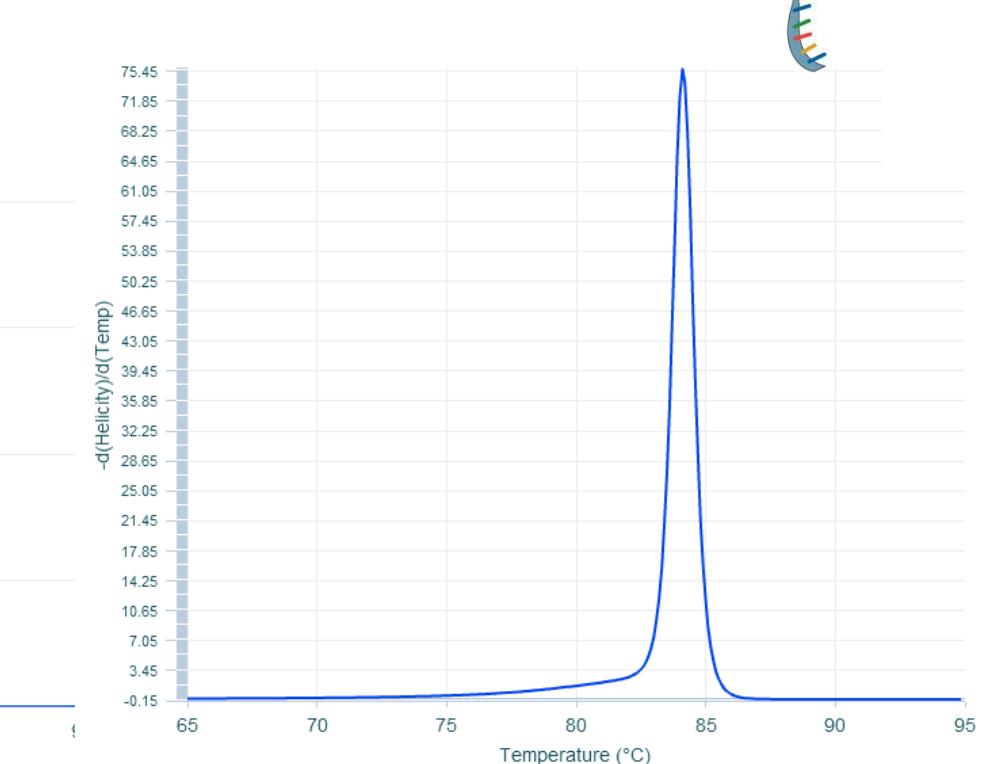
dsDNA = EvaGreen fluorescence



ssDNA = no fluorescence

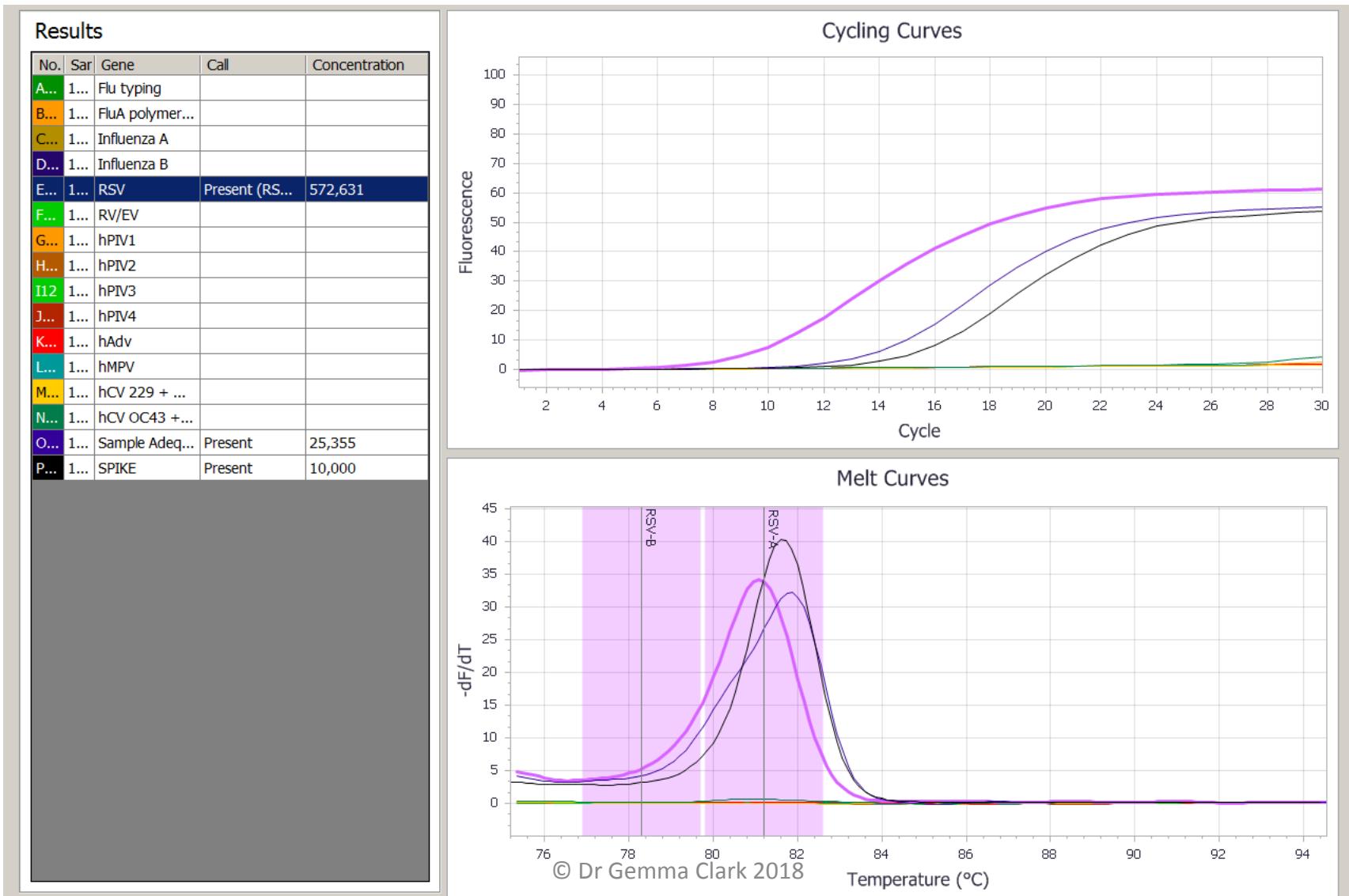


65°C → 90°C



Melt temperature specific to DNA sequence of amplicon (e.g. numbers of A, G, T and Cs)

Simplified results interpretation



Verification

- Parallel analysis
 - Concordant analysis with current assay using the same nucleic acid extract
- Retrospective analysis
 - Frozen samples (-80°C)
 - QCMD samples (<1 year old)

Target	Description	PPV	NPV
Influenza A	Typing assay H1 (2009) / H3	100%	100%
Influenza A	Influenza A polymerase	100%	100%
Influenza A	Influenza A matrix	100%	100%
Influenza B	Influenza B	100%	100%
RSV	Respiratory Syncytial virus	83%	100%
RV/EV	Rhinovirus and enterovirus	64%	100%
hPIV1	Human parainfluenza virus 1	100%	100%
hPIV2	Human parainfluenza virus 2	100%	98%
hPIV3	Human parainfluenza virus 3	100%	100%
hPIV4	Human parainfluenza virus 4	100%	100%
hAdv	Adenovirus group B C and E	100%	98%
hMPV	Human metapneumovirus	80%	100%
hCV 229 + HKU-1	hCV 229E and hCV HKU-1	100%	100%
hCV OC43 + NL63	hCV-OC43 and hCV-NL63	100%	100%

Workflow analysis

- Decreased complexity enabled reoptimisation of staff groups
- Decreased hands-on-time requirement enables increased multi-tasking (merging of extraction and PCR teams)

	In-house Assay	AusDiagnostics
Max batch size	16 (on two plates)	23
Max daily capacity (samples)	64	92
NA extract required (μ L)	50	10
Total Hands on Time (min)	17.69	13
Total time to result (min)	285	345
Complexity Index	110.4	79.3

Workflow analysis

- Decreased complexity enabled reoptimisation of staff groups
- Decreased hands-on-time requirement enables increased multi-tasking (merging of extraction and PCR teams)

Summer scenario - 1 run per day (22 samples, 1 positive control, 1 negative control)

	09:00	09:15	09:30	09:45	10:00	10:15	10:30	10:45	11:00	11:15	11:30	11:45	12:00	12:15	12:30	12:45	13:00	13:15	13:30	13:45	14:00	14:15	14:30	14:45	15:00	15:15	15:30	15:45	16:00	16:15	16:30	16:45	17:00	17:15	17:30
Run 1																																			

Summer scenario - 2 runs per day (45 samples, 1 positive control, 1 negative control)

	09:00	09:15	09:30	09:45	10:00	10:15	10:30	10:45	11:00	11:15	11:30	11:45	12:00	12:15	12:30	12:45	13:00	13:15	13:30	13:45	14:00	14:15	14:30	14:45	15:00	15:15	15:30	15:45	16:00	16:15	16:30	16:45	17:00	17:15	17:30
Run 1																																			
Run 2																																			

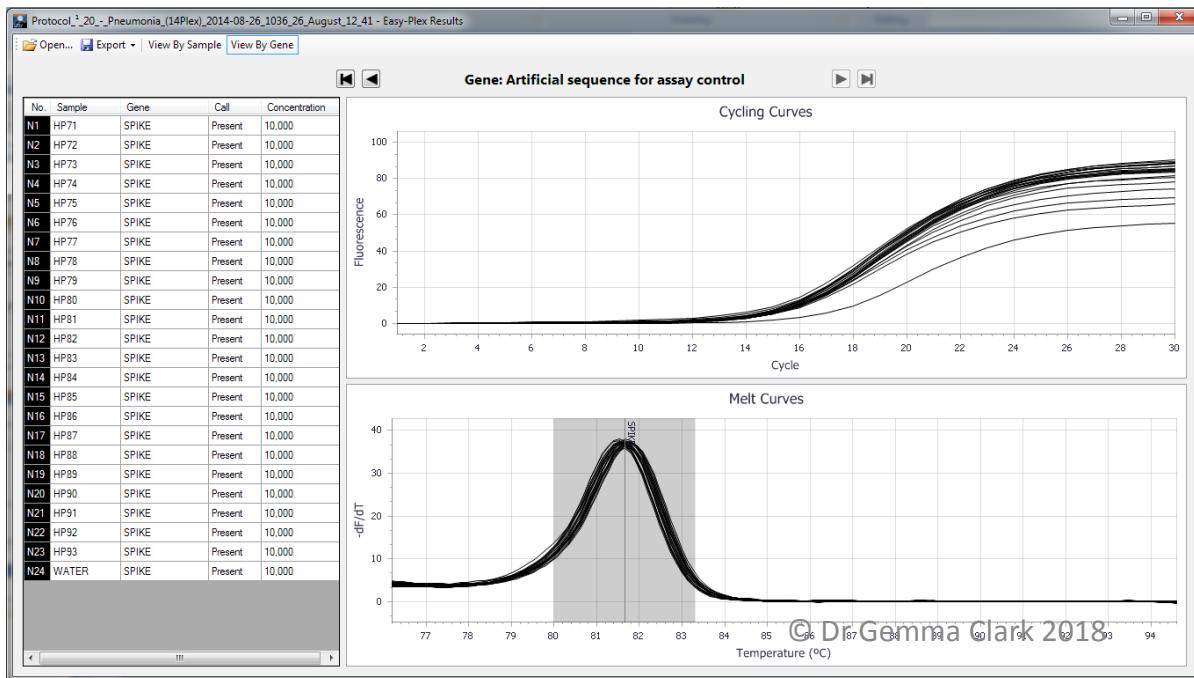
Winter scenario - 3 runs per day (68 samples, 1 pos control, 3 negative controls)

	09:00	09:15	09:30	09:45	10:00	10:15	10:30	10:45	11:00	11:15	11:30	11:45	12:00	12:15	12:30	12:45	13:00	13:15	13:30	13:45	14:00	14:15	14:30	14:45	15:00	15:15	15:30	15:45	16:00	16:15	16:30	16:45	17:00	17:15	17:30
Run 1																																			
Run 2																																			
Run 3																																			



Concentration?

- Semi-quantitative result (copies/10 μ l)
- Not strictly comparable to Ct
- Useful to monitor trends



Results

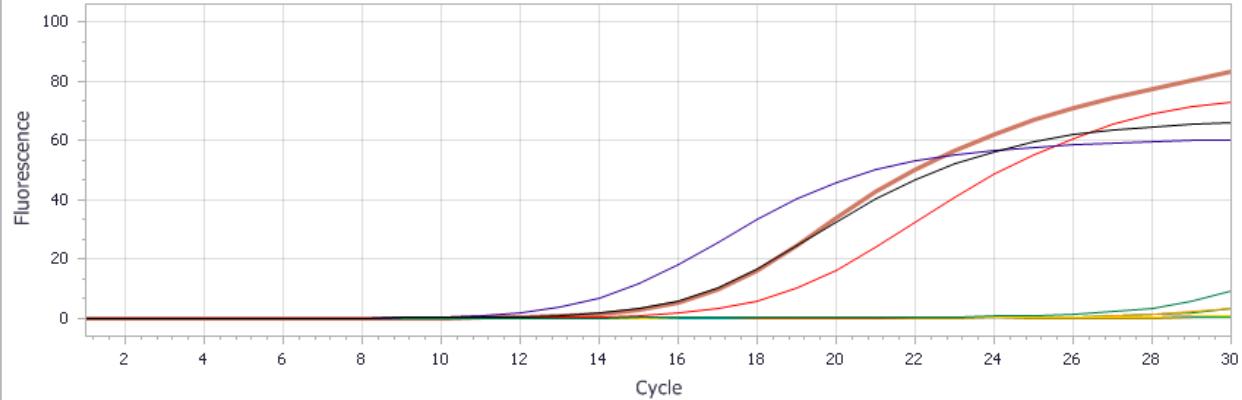
No.	Sar	Gene	Call	Concentration
A...	1...	Flu typing		
B...	1...	FluA polymer...		
C...	1...	Influenza A		
D...	1...	Influenza B		
E...	1...	RSV	Present (RS...	572,631
F...	1...	RV/EV		
G...	1...	hPIV1		
H...	1...	hPIV2		
I12	1...	hPIV3		
J...	1...	hPIV4		
K...	1...	hAdv		
L...	1...	hMPV		
M...	1...	hCV 229 + ...		
N...	1...	hCV OC43 +...		
O...	1...	Sample Adeq...	Present	25,355
P...	1...	SPIKE	Present	10,000

Mutation?!

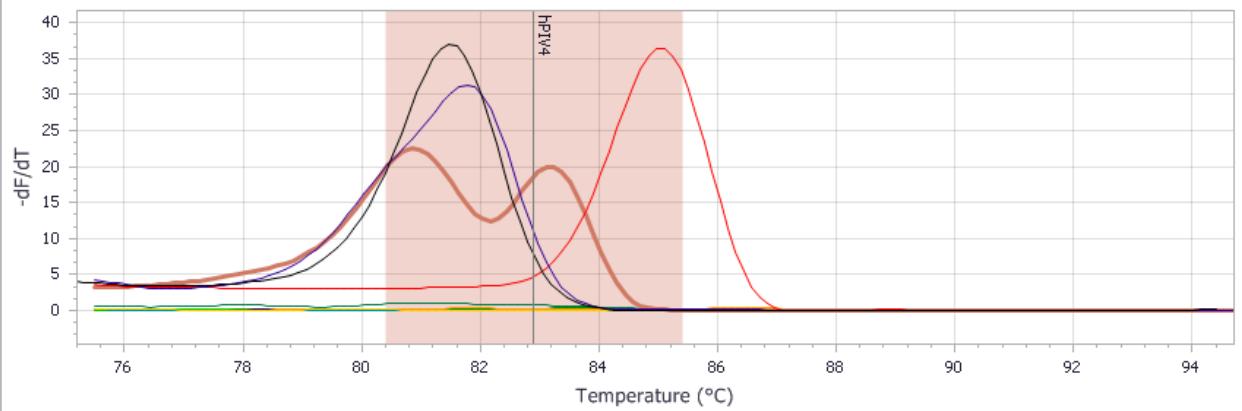
Results

No.	Sample	Gene	Call	Concentration
A5	600404	Flu typing		
B5	600404	FluA-polymerase		
C5	600404	Influenza A		
D5	600404	Influenza B		
E5	600404	RSV		
F5	600404	RV/EV		
G5	600404	hPIV1		
H5	600404	hPIV2		
I5	600404	hPIV3		
J5	600404	hPIV4		
K5	600404	hAdv	Present	2,722
L5	600404	hMPV		
M5	600404	hCV 229 + HKU-1		
N5	600404	hCV OC43 + NL63	Rejected	
O5	600404	Sample Adequacy	Present	49,011
P5	600404	SPIKE	Present	10,000

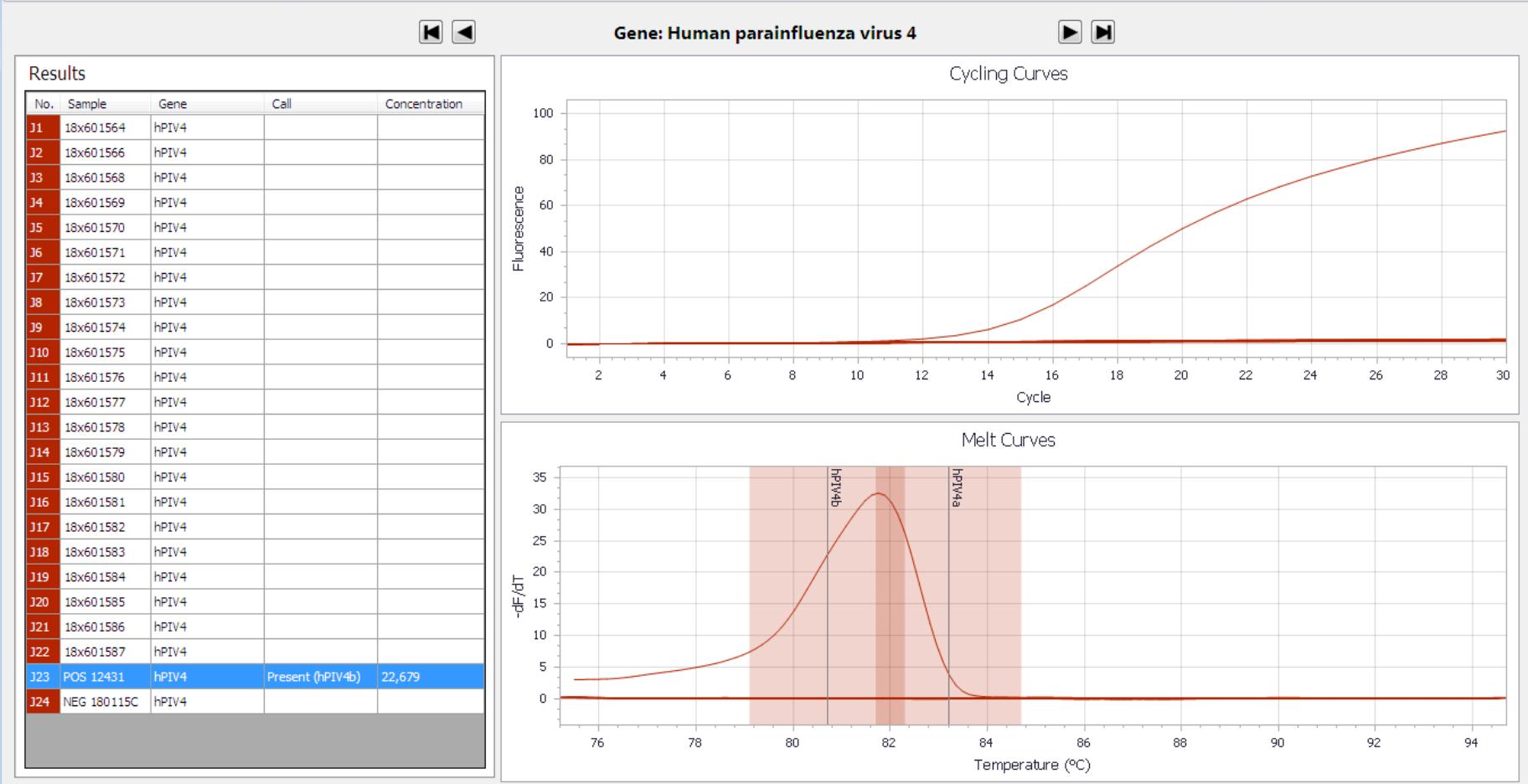
Cycling Curves



Melt Curves

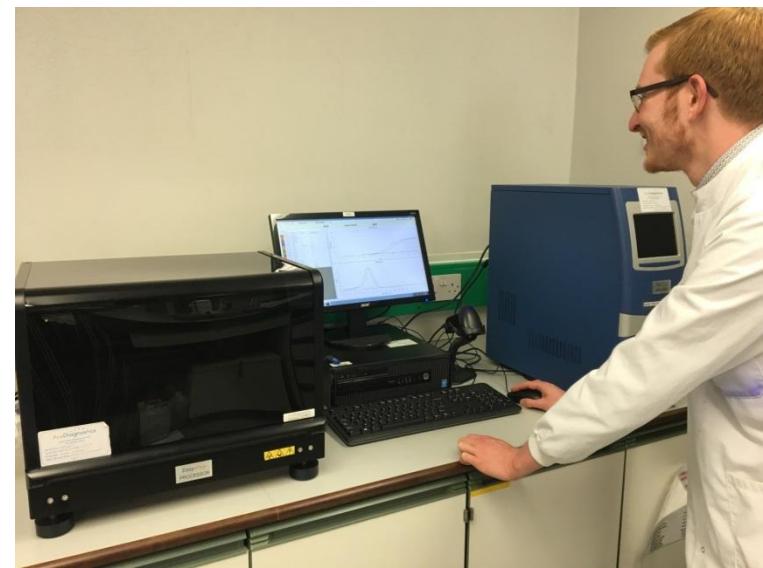


Mutation?!



RVP c.2018

- Maintenance of TAT despite 28% increase in workload annually
- Improved EQA performance
- Improved staff satisfaction
- Three analysers
- 6 runs per day



The Next Generation

- Further improvement to TAT required to optimise antiviral therapy
- Influenza working group
- Point of care testing
 - A&E
 - Respiratory Assessment Unit
- Roll out Winter 2018

