Molecular Diagnosis of Respiratory Infection

Malcolm Guiver

Clinical Scientist
Manchester HPA Northwest

Molecular Diagnosis of Respiratory Infection

- Outline the reasons for switching from cell culture to a molecular approach.
- Describe the developed assays
- Compare the detection rates for cell culture and MD methods
- Discuss some of the challenges produced in clinical interpretation
- Outline future developments for expanding the repertoire

Why switch to PCR?

- Cell culture
  - Slow, insensitive, cell line dependent
- Increasing demand for quicker results.
  - Increasing numbers of vulnerable immunocompromised patients
  - Increasing treatment options. Reduce unnecessary antibiotic use
- Virus discovery programmes identifying new pathogens that cannot be cultured
- Expand service and increase detection rates
  - Provide better epidemiological data

Assay Development

- Real-time PCR based assays developed at Manchester from 2002.
- Co-ordinated developments and evaluations through Molecular Diagnostic Forum, avian ‘flu roll out and CVN initiated in 2006
- Alternative strategies eg microarray, microbead Luminex are available
- Quantification increasingly important in clinical interpretation.

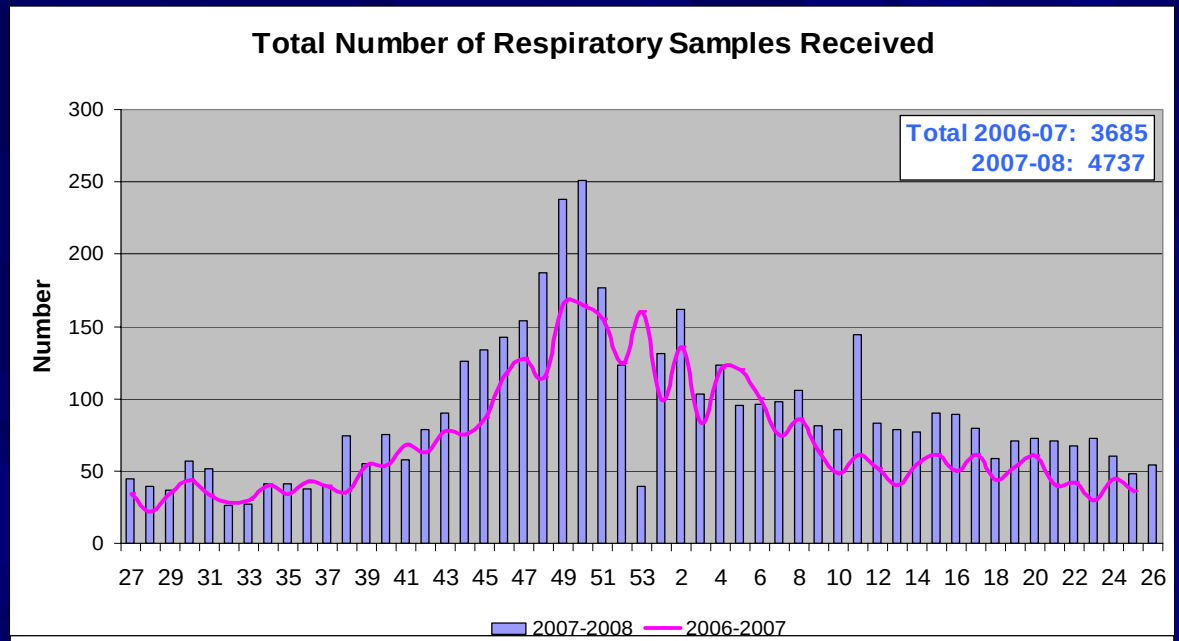
Multiplex PCR assays

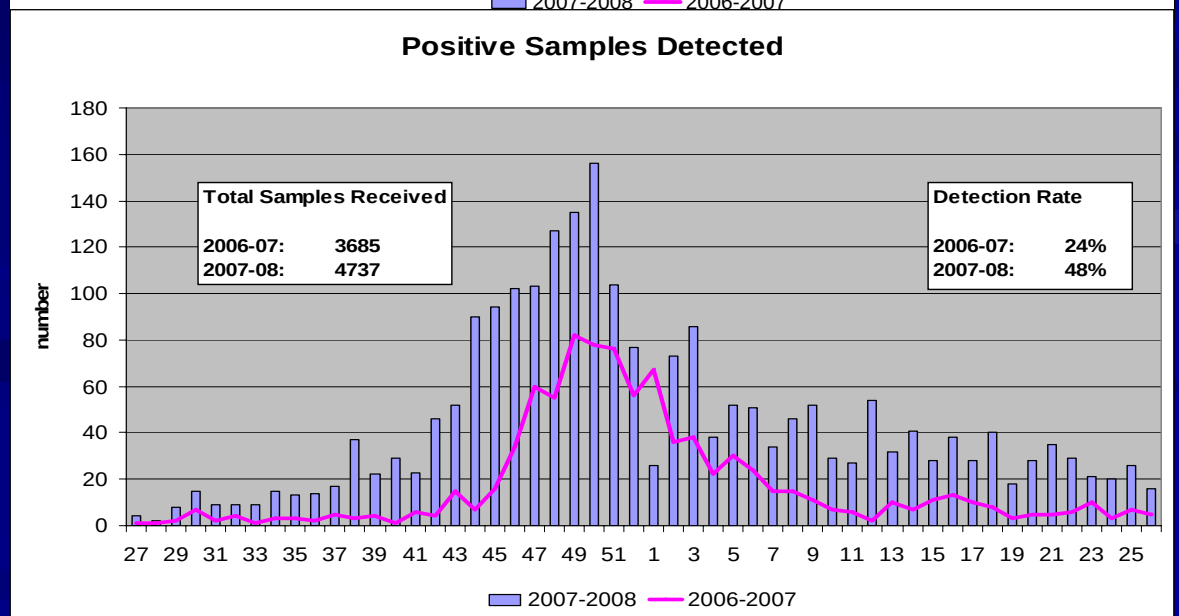
4 multiplex reactions for 9 targets

Flu A
Flu B
RSV A and B
Paraflu 1,2,3
Rhinovirus
Metapneumovirus
Adenovirus

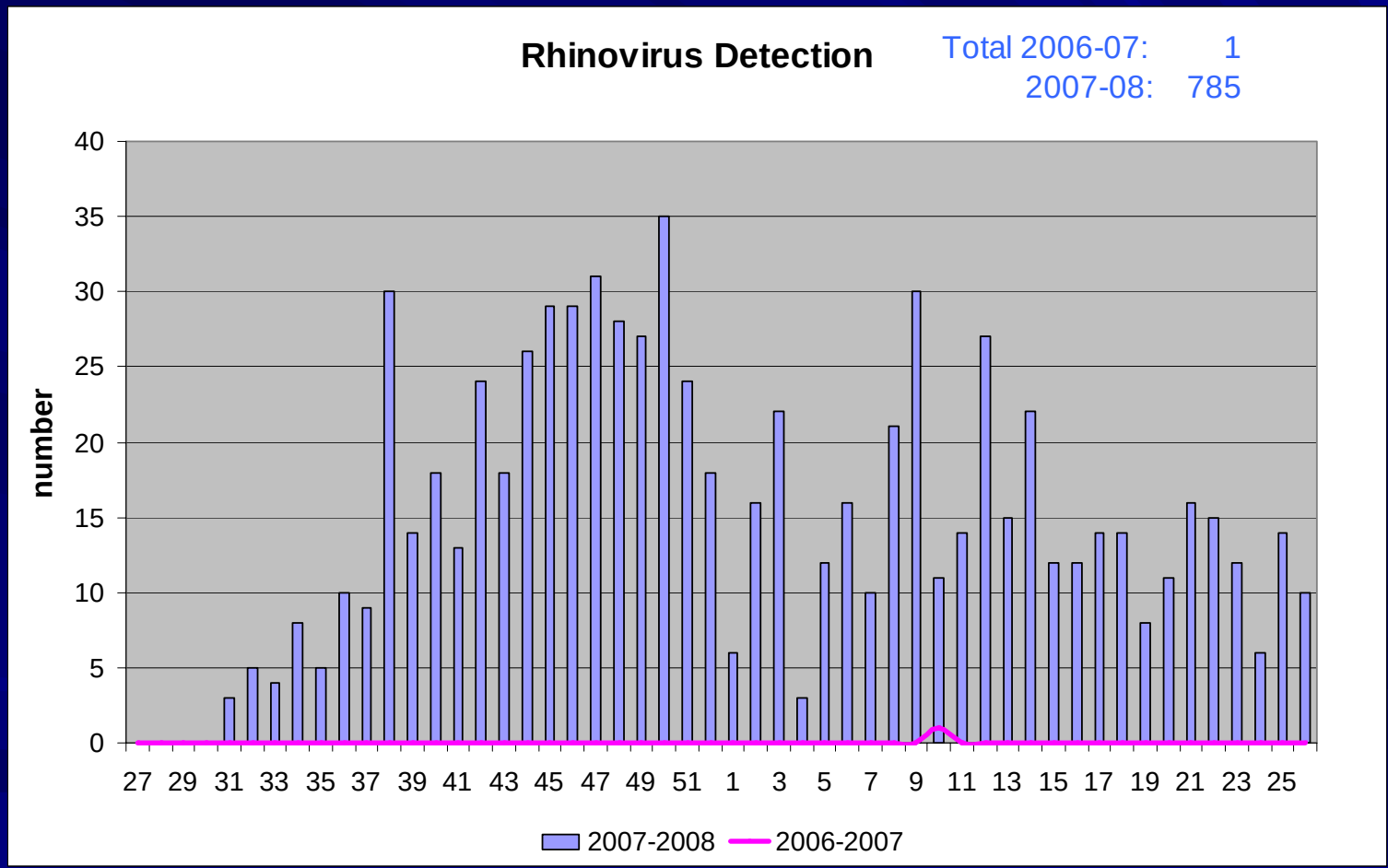
- From September 2007 a 24 hour PCR respiratory diagnostic service has been offered replacing cell culture.
- High throughput automated extraction and real-time detection essential for a robust service with 24 hour turn around time

Was it worth it?

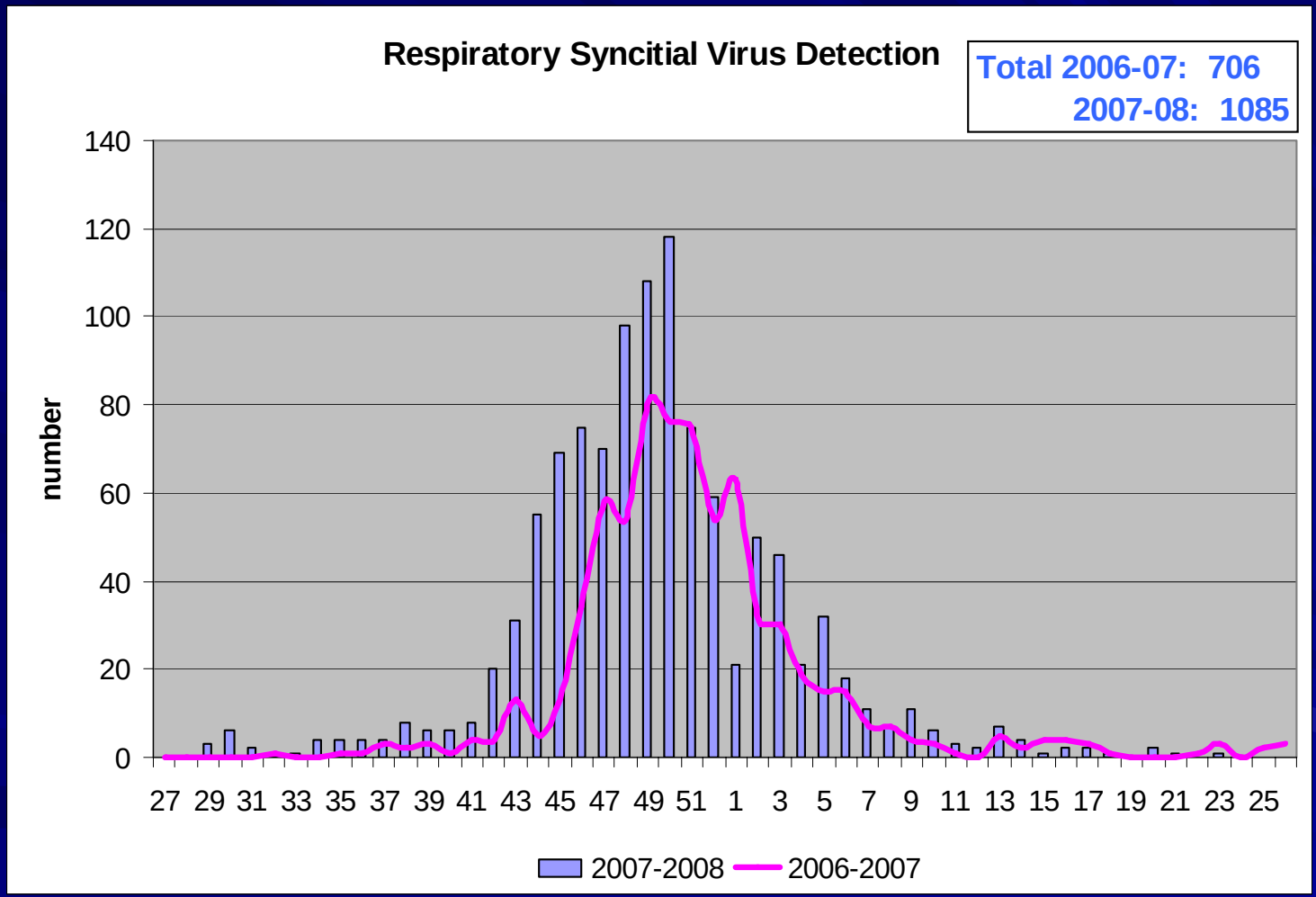




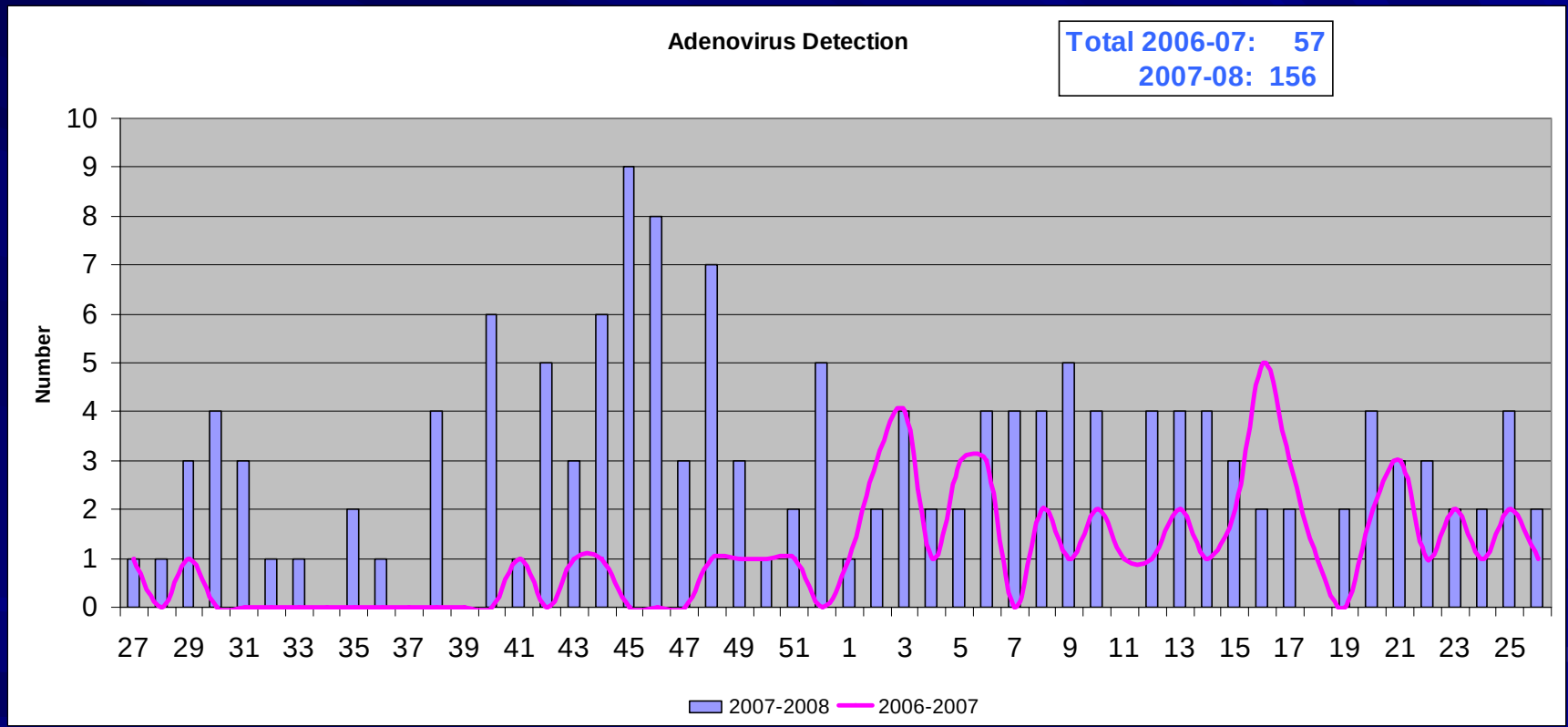
Rhinovirus


Rhinovirus Detection
Total 2006-07: 1
2007-08: 785
number
40
35
30
25
20
15
10
5
0
27 29 31 33 35 37 39 41 43 45 47 49 51 1 3 5 7 9 11 13 15 17 19 21 23 25
2007-2008
2006-2007

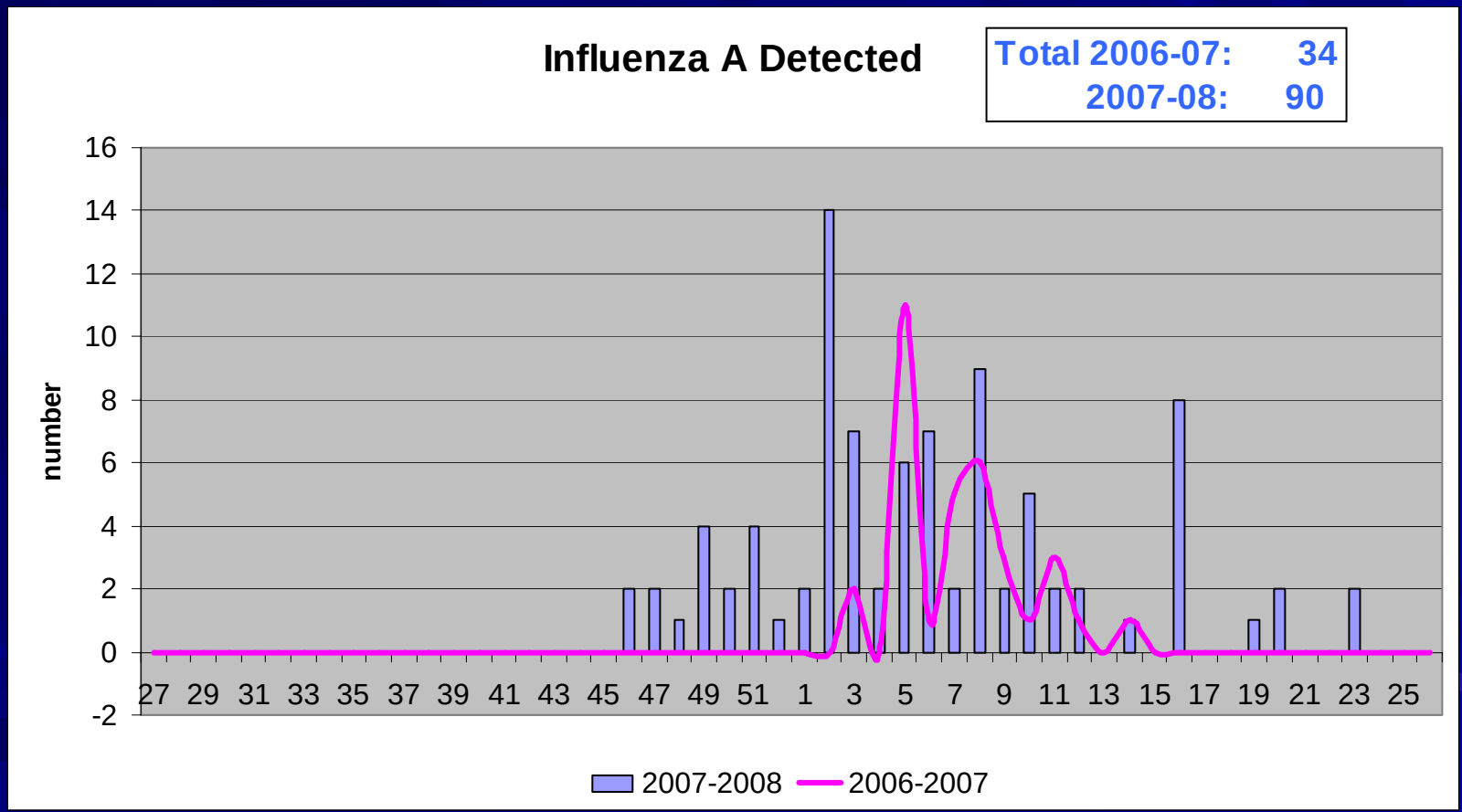
RSV


Respiratory Syncitial Virus Detection
Total 2006-07: 706
2007-08: 1085
number
140
120
100
80
60
40
20
0
27 29 31 33 35 37 39 41 43 45 47 49 51 1 3 5 7 9 11 13 15 17 19 21 23 25
2007-2008
2006-2007

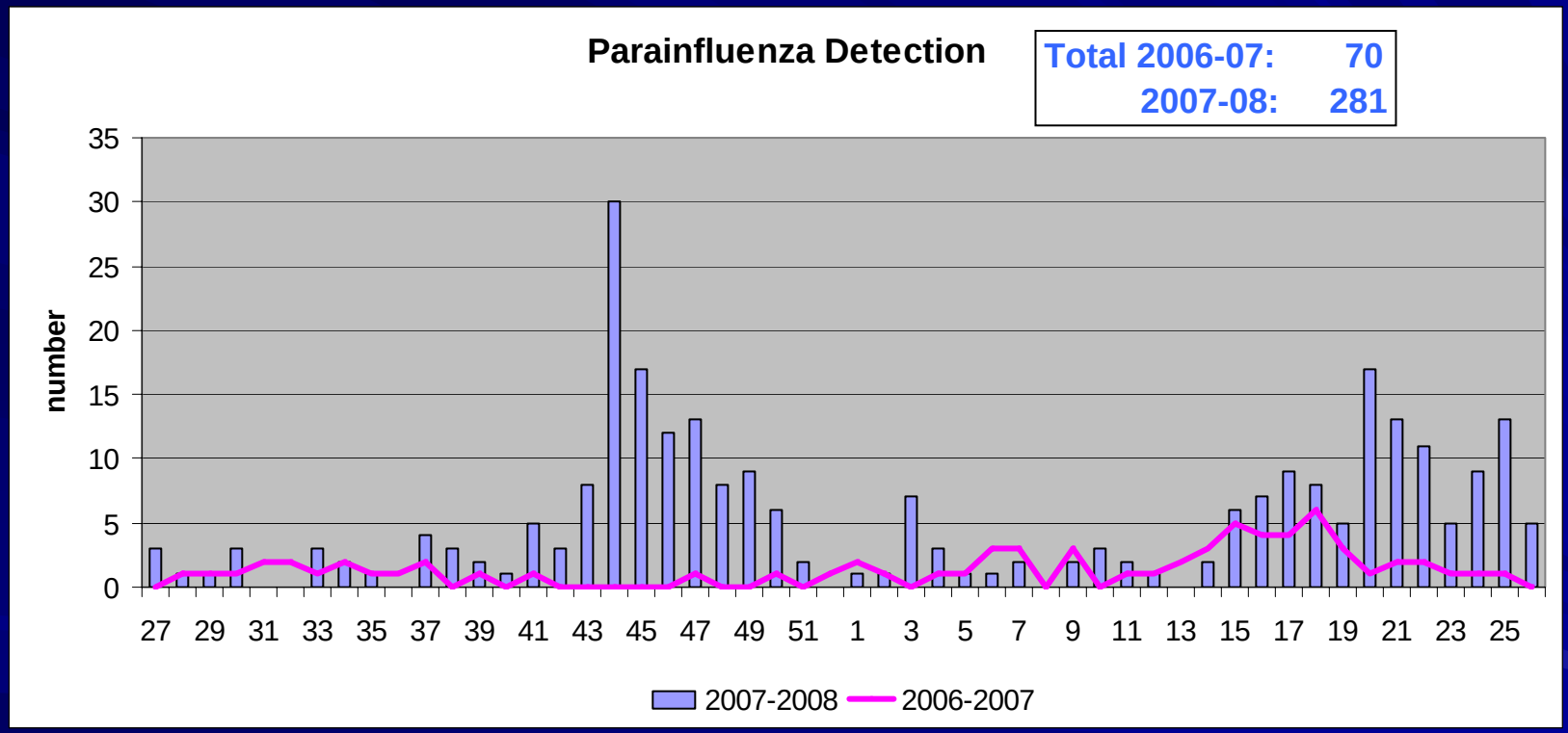
Adenovirus


Adenovirus Detection
Total 2006-07: 57
2007-08: 156
Number
10
9
8
7
6
5
4
3
2
1
0
27 29 31 33 35 37 39 41 43 45 47 49 51 1 3 5 7 9 11 13 15 17 19 21 23 25
2007-2008
2006-2007

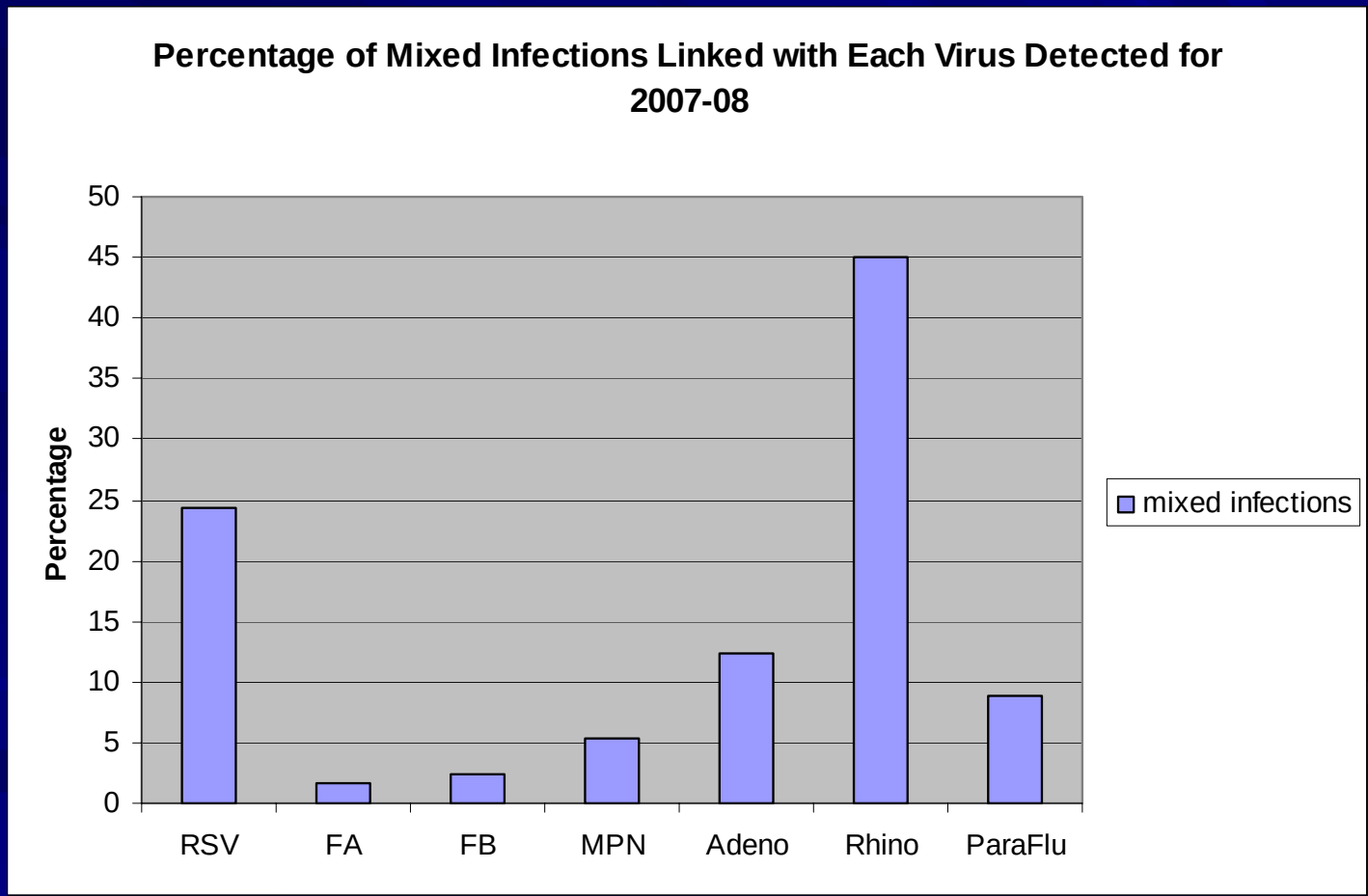
Influenza A


Influenza A Detected
Total 2006-07: 34
2007-08: 90
number
16
14
12
10
8
6
4
2
0
-2
27 29 31 33 35 37 39 41 43 45 47 49 51 1 3 5 7 9 11 13 15 17 19 21 23 25
2007-2008
2006-2007

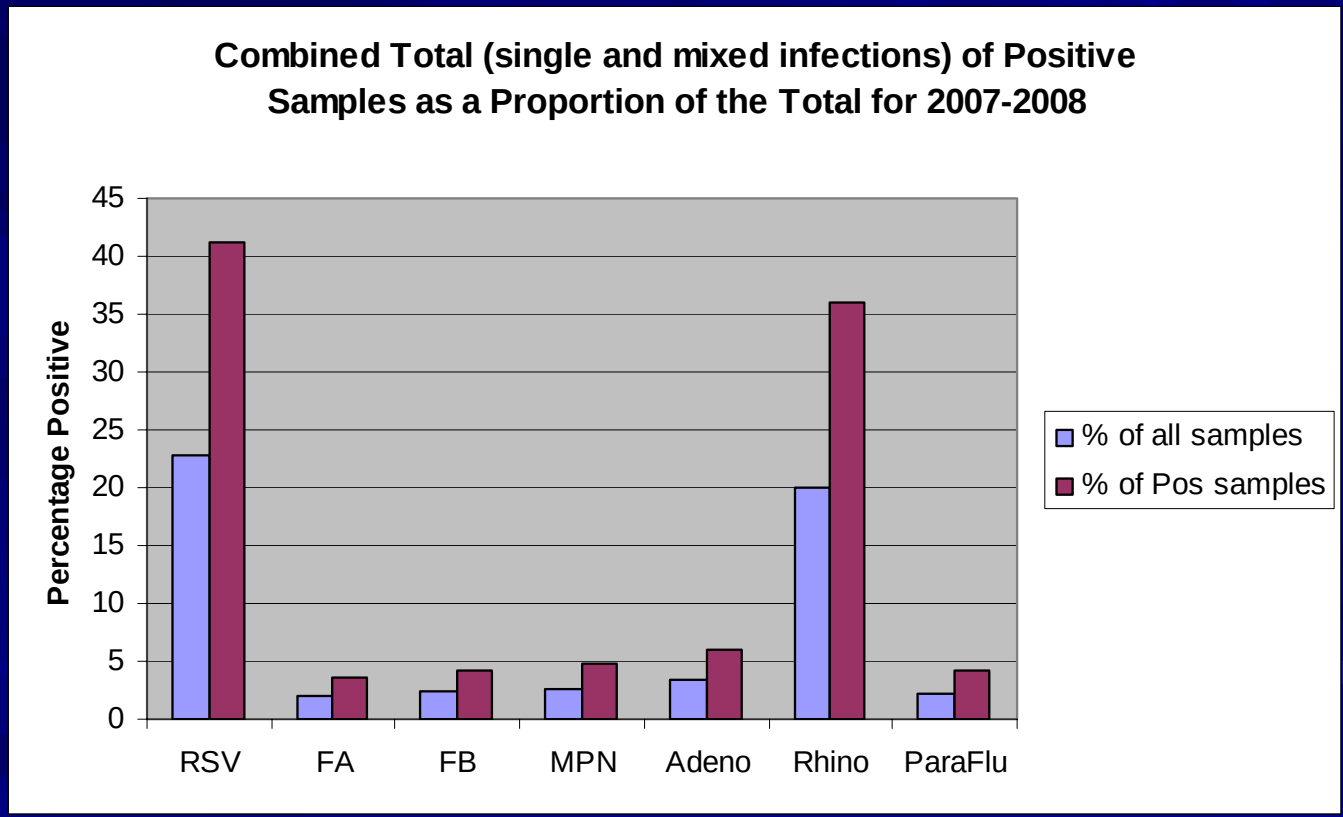
Parainfluenza



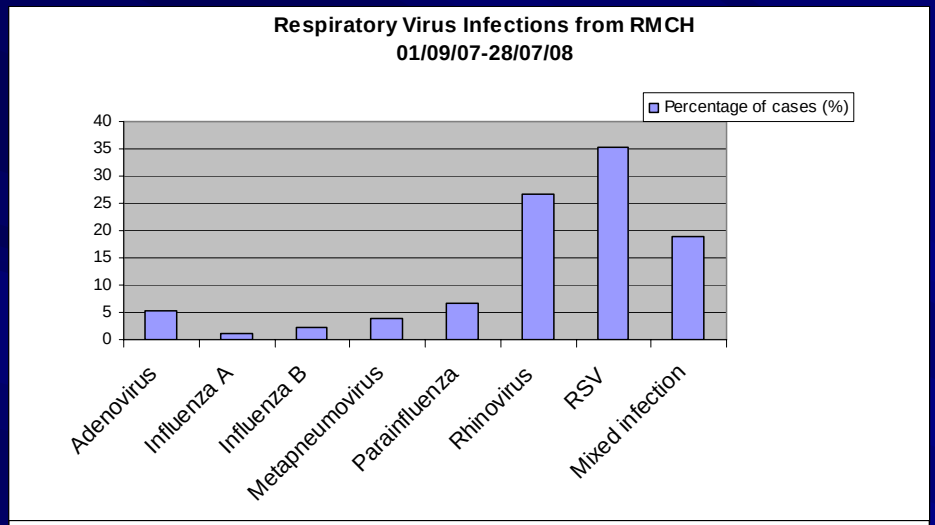
37% of Positive Samples had 2 or More Viruses Present

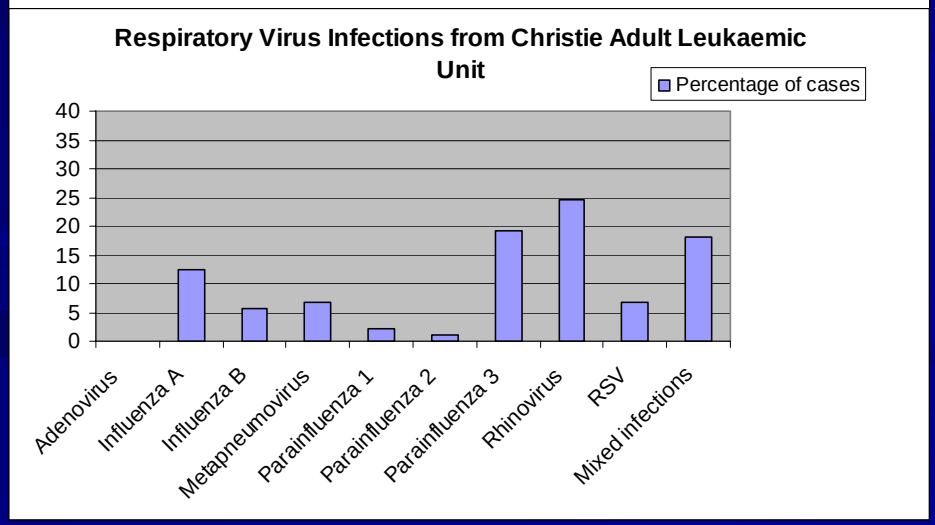


Summary of Positive Samples detected by PCR

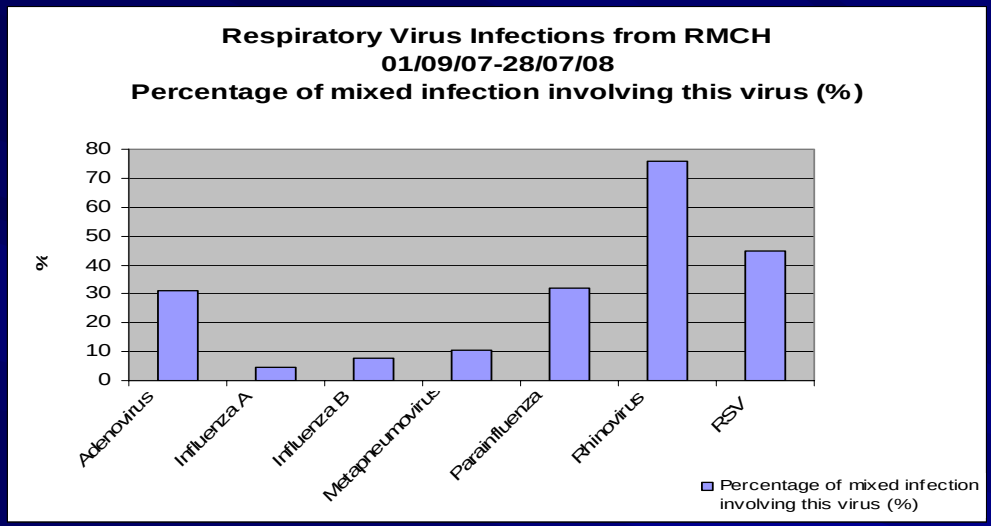


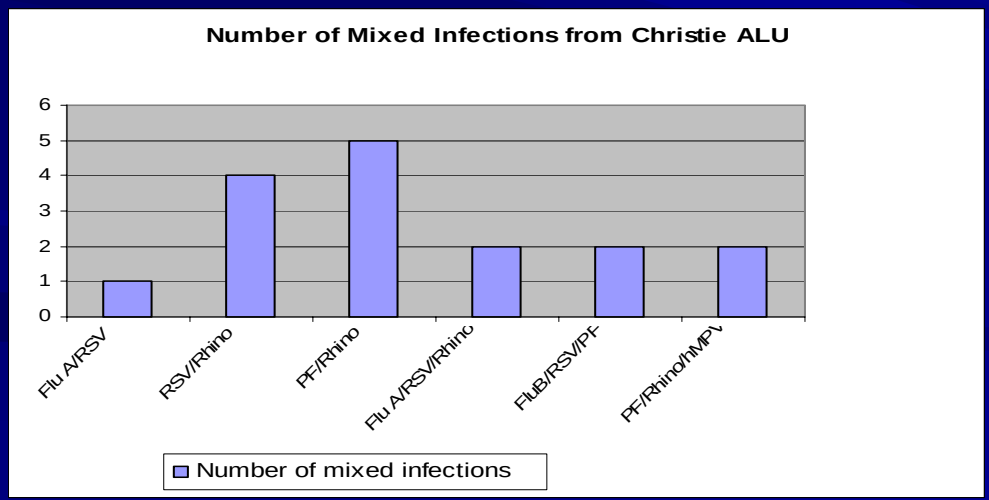
Comparison of Infections in Adults and Children 01/09/07-28/07/08





- RSV more common in children
- Proportion of mixed infections similar
- No adenovirus infections in adults-primary infections in children occurring





- Rhinovirus features in the majority of mixed infections-75% mixed infections included rhinovirus in samples from RMCH

Significance of Mixed Infections

- Impact on severity of symptoms?
- Sample type may influence recovery
- Quantification can be beneficial
- May be useful in cohorting patients

RSV Outbreak on Neonatal Surgical Unit

Date	Patient 2	
	I.F. Result	PCR Cycle Threshold
10.12.07	INS	22.2
11.12.07	INS	20.1
12.12.04	POS	18.3
13.12.07	POS	20.0
14.12.07	POS	25.0
15.12.07	POS	23.0
16.12.07	POS	21.0
19.12.07	INS	37.4
31.12.07	NEG	36.7
10.01.08	POS	29.8
14.01.08	NEG	41.4
18.01.08	NEG	NEG

RSV Outbreak

Date	Patient 3	
	I.F. Result	PCR Cycle Threshold
27.11.07	INS	27.6
29.11.07	POS	24.1
01.12.07	POS	24.4
03.12.07	INS	23.1
05.12.07	NEG	27.1
06.12.07	POS	27.7
08.12.07	NEG	25.8
09.12.07	POS	29.7
10.12.07	INS	30.6
11.12.07	INS	28.0
12.12.04	NEG	29.7
13.12.07	NEG	32.0
14.12.07	NEG	33.0
15.12.07	NEG	31.0
16.12.07	NEG	Not Tested
17.12.07	POS	Not Tested
18.12.07	POS	27.0
19.12.07	INS	29.9
20.12.07	INS	31.4
21.12.07	POS	28.0
22.12.07	POS	25.6
23.12.07	NEG	31.8
24.12.07	NEG	38.9
27.12.07	NEG	39.2

RSV Outbreak

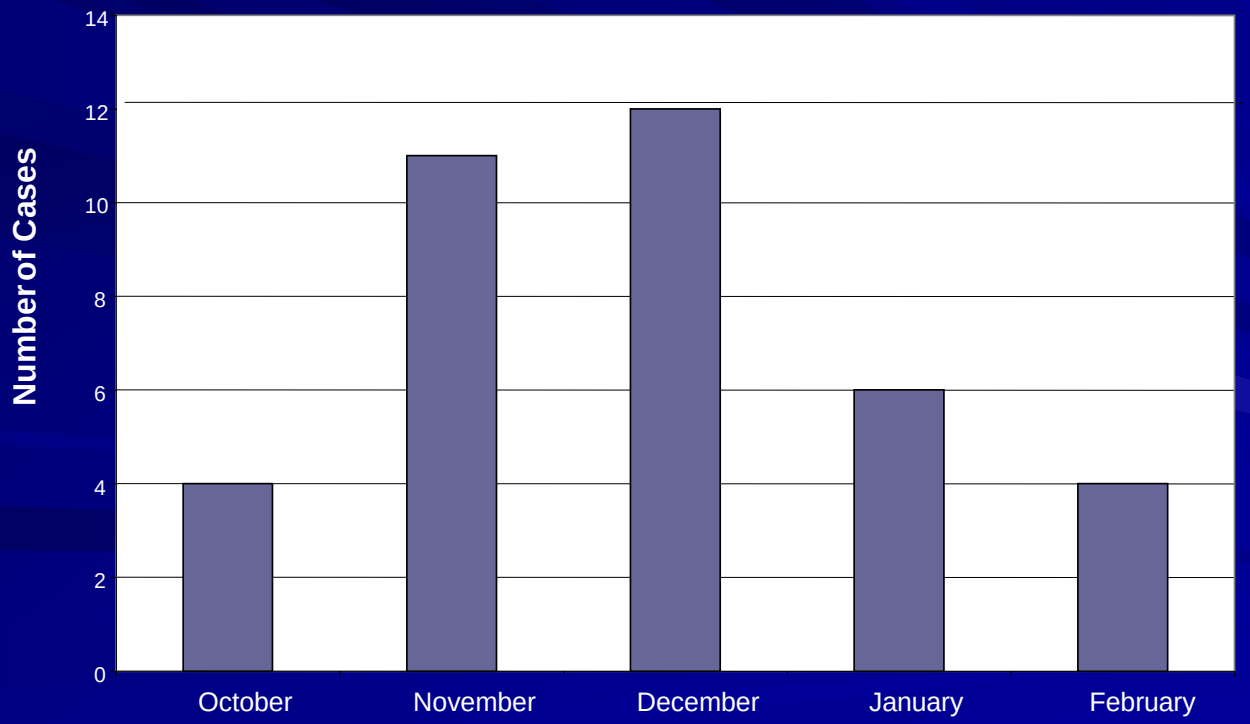
- PCR proved to be more sensitive than I.F.
- I.F. testing was unreliable
- PCR results remained positive for extended periods
- Potential infectivity: clinical symptoms and PCR result

Future Developments

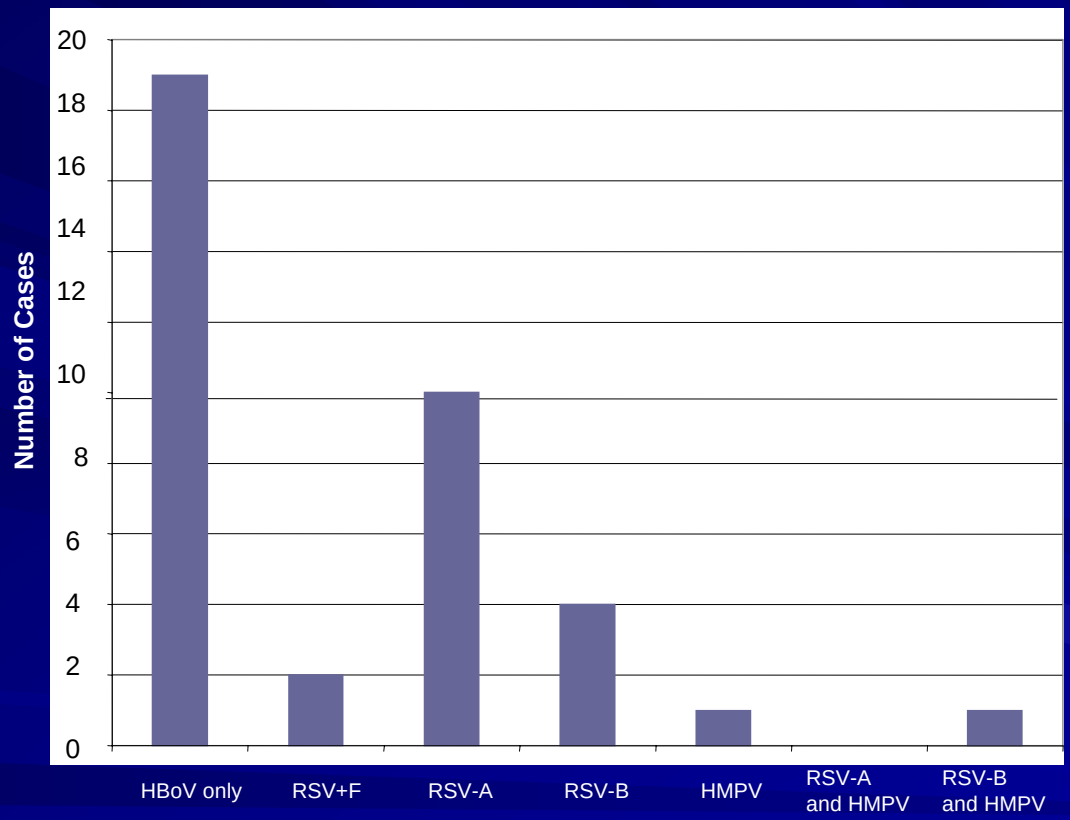
- New assays
- Bocavirus
- Coronavirus 229E, NL63, OC43
- Mycoplasma pneumoniae
- and other atypical pneumonia infections

Bocavirus Detection

- New parvovirus described Allander *et al* in 2005 associated particularly with respiratory disease but also linked with gastrointestinal disease in children.
- From October 2006 to February 2007, 369 nasopharyngeal aspirates (NPA) of hospitalised patients with respiratory tract disease were tested by Taqman PCR for bocavirus infection
- **37 (10.02%) samples were positive**



Mixed Infections with Bocavirus positive samples



48% mixed infections

Is Bocavirus Causing Disease?

- Commonly found as a co-infection
- May be shed persistently
- May be reactivated by another infection
- Quantitative PCR more predictive of disease
- Primary infection associated with viraemia in <3 year olds
- Serology promising
- Is molecular detection in respiratory samples the best approach?

Virus Discovery and Clinical Significance

- Bocavirus? WU and KI?
- Pathogen of passenger?
- Significance in different patient groups with symptoms more difficult to establish
  - Kate Templeton and Peter Simmonds J Clin Virol. 2007 Dec;40(4):307-11. Epub 2007 Nov 7. No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease

Summary

- Molecular diagnosis significantly improves detection rate compared to cell culture
- Non-invasive nose and throat swabs can be taken
- All results can be reported within 24 hours or less (up to 2 weeks by culture)
- The large number of mixed infections was a significant feature of PCR detection
- Some indication of relative quantity of mixed infections is important in interpreting results
- Newly discovered pathogens can be easily incorporated into screening assay
- Increase in sensitivity presents new challenges in managing infection control issues (correlation of detection and potential for transmission)