point-of-care test in Virology

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point-of-care test (POCT)

- **Definition:**
  - **an analytical or diagnostic test undertaken in a setting distinct from a normal hospital or non-hospital laboratory**
  - **performed by a health care professional or non-medical person**
    - **bedside testing, near-patient testing, physician's office testing, extra-laboratory testing, decentralised testing, offsite, ancillary, or alternative site testing**
- **distinguishing POCTs from other rapid diagnostic methods**
  - **is able to be performed by non-laboratory trained staff and without the use of complicated and poorly transportable equipment (e.g. microscopes, centrifuges).**
  - **It can be also used by laboratoriesas a rapid alternative to other "usual" laboratory tests, or where testing facilities are limited**

POCT application

- **many areas of clinical medicine:**
  - **biochemical assays (e.g. glucose, sodium, cardiac enzymes, and cholesterol)**
  - **haematological assays (e.g. haemoglobin, prothrombin/INR, ESR, and HbA1C)**
  - **Hormonal assays (e.g. bHCG, LH, and FSH) and drug assays (e.g. alcohol, amphetamines, and cannabinoids).**
  - **Infectious diseases**
    - **to detect antigens (e.g. of *Streptococcus pyogenes, Streptococcus pneumoniae, Legionella pneumophila, influenza* viruses, and respiratory syncytial virus)**
    - **pathogen-specific antibodies (e.g. HIV/HCV antibodies).**

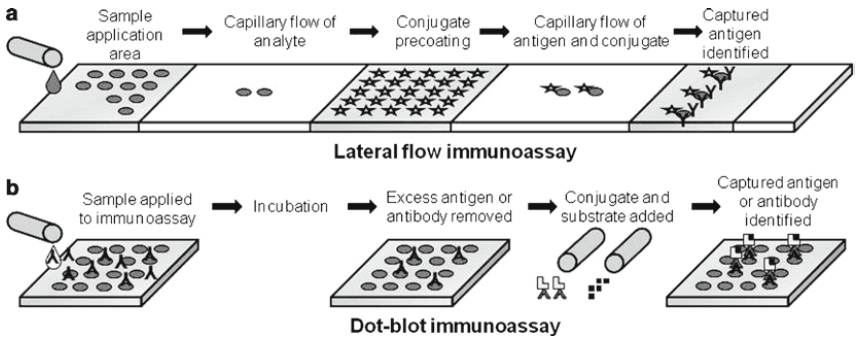
key features of POCT

- Is highly sensitive and specific
- Gives a result that improves treatment (and reduces cost) by reducing inappropriate treatment and hospitalisation
- Can be done rapidly (15–30 min)
- Is simple to perform and interpret by non-laboratory personnel
- Contains internal controls to help assure the validity of results
- Does not require expensive or elaborate equipment
- Has temperature stable components that allow easy and prolonged storage
- Is relatively inexpensive

- **Test Turn-around Time and Clinical Turn-around Time**

Test Format

Routine Platforms



(a) Antigen detection by lateral flow immune assay or immuno-chromatographic test or ICT
(b) antibody detection by dot-blot immunoassay

Molecular platforms with point-of-care testing potential

Digital medicine:

A laboratory in your pocket

POCT for Respiratory Viruses

- **Methods to identify respiratory viruses:**
  - **a) viral culture with virus confirmation with monoclonal antibodies**
  - **b) antigen detection by staining of clinical specimens with monoclonal fluorescent antibodies**
  - **c) rapid antigen/genome detection by POCT**
  - **d) direct viral genome detection by nucleic acid testing (NAT)**
  - **e) acute and convalescent serology**
- **POCTs for respiratory viruses are available for some respiratory viruses: influenza, RSV, adenovirus**
- **One of the major applications of POCTs has been in the rapid detection of respiratory pathogen in community and hospital respiratory infections.**
- **The diagnosis of respiratory viruses is important to enable early infection control procedures and both timely and appropriate treatment wherever available.**

Influenza Virus

- **Many POCTs now distinguish both influenza A and B in the same test.**
  - **Although type-specific influenza POCTs exist, subtyping is not possible.**
    - **subtyping is required to determine optimal treatment strategies**
  - **POCTs do not perform equally for all influenza viruses**

Test	Manufacturer	Specimen	Sensitivity (%)	Specificity (%)	Time (min)
BinaxNOW® influenza A & B	Binax, Scarborough, ME, USA	NPA[a], NPS, NW	58–82	92–100	15
Directigen flu A + B	Becton-Dickinson, Cockeysville, MD, USA	BAL, NPA, NPS, NW, TS	81–86	91–99	15
Directigen flu A + B/EZ	Becton-Dickinson, Cockeysville, MD, USA	NPA, NW, TS	69–86	86–100	15
Flu optical immunoassay A/B	Thermo Electron, Waltham, MA, USA	NPA, NPS, TS, sputum	62–88	52–80	15
QuickVue® Influenza A + B	Quidel Corporation, San Diego, CA, USA	NPA[a], NW, nasal swab	72–82	96–100	10
Xpect fluA and B	Remel Inc, Lenexa, KS, USA	NPS, NW, TS	83–100	100	15
ZstatFlu-II™	ZymeTx Inc, Oklahoma City, OK, USA	NPA[a], TS	50–88	83–100	30

BAL Bronchoalveolar lavage, *NPA* nasopharyngeal aspirate, *NPS* nasopharyngeal swab, *NW* nasal wash, *TS* throat swab
[a]CLIA-waived

Reduced sensitivity of RIDT (QuickVue) for newly circulating pandemic influenza virus

Parameter	H1N1 09 (n = 174)[a]	A/H3 (n = 88)[a]	Non-H1N1 09 (n = 97)[b]
No. of samples RAT positive	93[a]	68[a]	72
No. of samples RAT negative	81	20	25
Sensitivity (%)	53.4	77.2	74.2
Specificity (%)	100	100	100
PPV[c] (%)	100	100	100
NPV[d] (%)	76.2	92	90.2

[a] Includes two samples that were coinfected with H1N1 09 and influenza A/H3.

[b] Includes 88 influenza A/H3 and 9 others (3 seasonal influenza A/H1N1 and 6 untypeable).

[c] PPV, positive predictive value.

[d] NPV, negative predictive value.

Kok J Clin Microbiol 2010

Influenza Virus

Variation in test sensitivity

- Epidemiology and Disease prevalence (e.g. during seasonal outbreaks), Specimen type, and Treatment
  - Higher sensitivity is observed in children compared with adults
  - when nasopharyngeal aspirates are compared with nasal or throat swabs
  - when specimens are collected early in the illness (when viral shedding is highest) and prior to antiviral therapy

Rate of detection of respiratory viruses differs according to age and samples tested

	Recovery using flocked swabs	Recovery using comparator	p values and Δ in CT values (flocked swab – comparator)
DeByle J Virol Methods 2012 (n=314 children < 3 years)	79 – 89% (nasopharyngeal)	69 – 94% (nasal wash)	p=0.069 – 1.0 0.6 – 7.0
Munywoki J Clin Microbiol 2011 (n=299 children < 13 years)	89.6% (nasopharyngeal)	79.2% (nasal wash)	p=0.0043 -1 – -2
Hernes Eur J Clin Microbiol Infect Dis 2011 (n=223 adults)	78% (nasopharyngeal)	63% (oropharyngeal flocked swabs)	p <0.01 -5.75

Sensitivity of antigen tests stratified according to age groups in 2009 (n=2274)

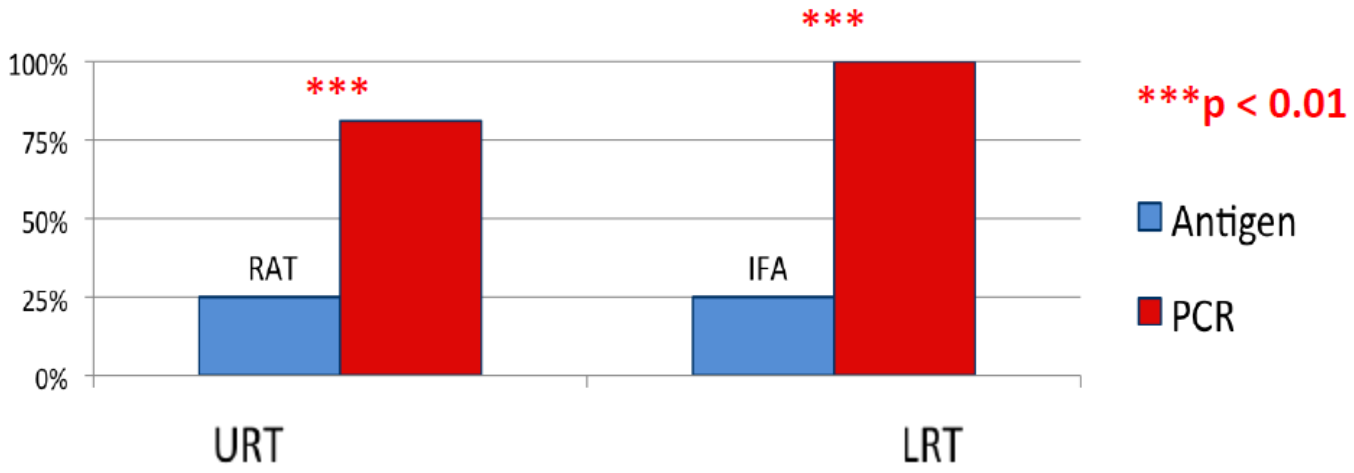
Age group	RIDT		IFA	
	pH1N1	Non-pH1N1	pH1N1	Non-pH1N1
0-1 years (n=65 RIDT, 46 IFA)	87.5%	100%	86.7%	90%
2-5 years (n=61 RIDT, 25 IFA)	70%	87.5%	100%	100%
6-15 years (n=160 RIDT, 25 IFA)	69.3%	71.9%	86.7%	83.3%
≥16 years (n=1503 RIDT, 389 IFA)	42.4%	72.1%	39.8%	56.7%

Kok ASID 2010

Important to test lower respiratory tract in critically ill (adult) patients with influenza

RIDT (QuickVue) and IFA vs NAT

- 21 patients with severe A(H1N1)pdm09 infection requiring respiratory support with paired URT / LRT samples
- Nose and/or throat swabs: RIDT
- BAL/mini BAL: IFA
- All samples: NAT



Blyth N Engl J Med 2009

Advantages of influenza virus rapid identification

advantages over "traditional" tests with longer TAT

I. **antigen detection by staining of clinical specimens with indirect fluorescent antibodies (laboratory TAT ≈ 3–4 h)**

II. **specific influenza nucleic acid detection (laboratory TAT ≈ 12–24 h)**

- **effective therapy with Antiviral agents**
  - **Timely diagnosis is required;** during 48 hrs of symptom initiation
  - **reducing duration and severity of symptoms, secondary complications, and fatality rates**
- **reduced investigations and antibiotic use, shorter admission time, and less health care costs**
- **The portability of POCTs can assist in the diagnosis of institutional or community influenza outbreaks.**
  - **The rapid identification of influenza enables early public health intervention and therapy and may influence the nature of an influenza outbreak**

Novel antiviral agents for respiratory viruses

Virus	Existing agents	Novel agents in development
Influenza	Amantadine, Rimantidine Zanamivir, Laninamivir, Oseltamivir, Peramivir Favipiravir	DAS181 (Fludase®) CR6261 CR8020 AVI-7100 VX-787
RSV	Ribavirin Palivizumab Motavizumab	GS-5806 ALS-008176 RI-001 ALN-RSV01 TMC353121 MDT-637 ALX-0171

Virus	Existing agents	Novel agents in development
Parainfluenza virus	-	DAS181 (Fludase®)
Rhinovirus	-	Vapendavir (BTA798) SNG001 (IFN-β)
Adenovirus	Cidofovir	Brincidofovir (CMX001)

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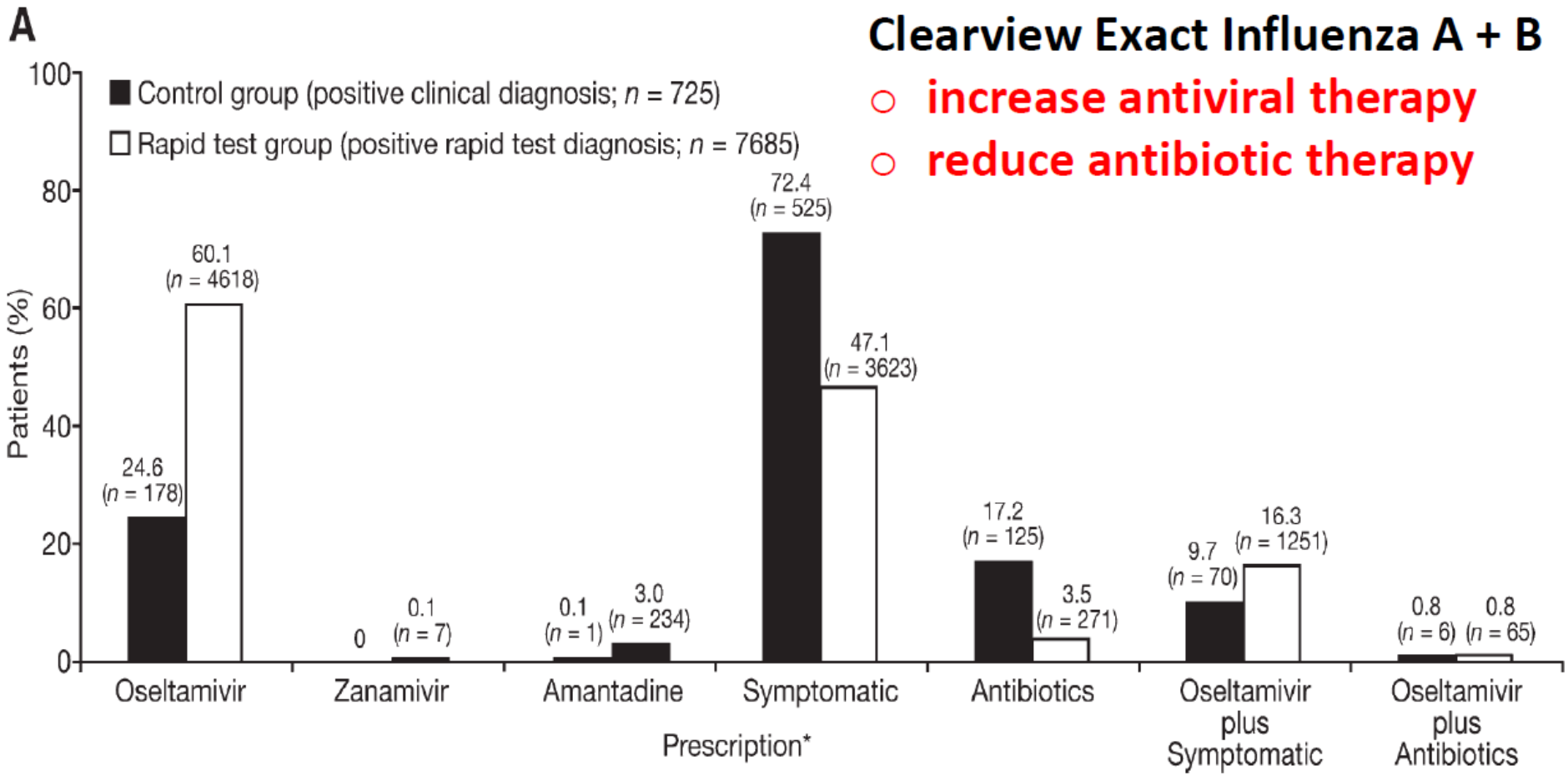
A positive RIDT alters outpatient pediatrician practices in ILIs



Clearview Exact Influenza A + B

- **increase antiviral therapy**
- **reduce antibiotic therapy**

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Jennings IORV 2009

Impact of RIDT

	MD aware RIDT positive (n=96)	MD unaware RIDT positive (n=106)	P value
CBC	0	13 (12%)	<0.001
BC	0	11 (13%)	<0.001
Urinalysis	2 (2%)	12 (11%)	0.011
CXR	7 (7%)	26 (25%)	0.001
Charge/patient	$15.65	$92.37	<0.001
Antibiotic prescription	7 (7%)	26 (25%)	<0.001
Antiviral prescription	18 (19%)	7 (7%)	0.02
Mean time from exam to discharge	25 minutes	49 minutes	<0.001

Bonner Pediatrics 2003

Influenza Virus poor sensitivity

- **Reliance on POCTs will miss a significant proportion of those infected with influenza.**
  - **unacceptably poor sensitivity, especially in adults where it is around 50% for influenza and lower for RSV**
  - **This is particularly important in the high-risk patients, but less so when multiple patients are tested during an outbreak**

meaning that they cannot be used to rule out infection

	Turnaround times (minutes)	Sensitivity	Specificity
Lateral flow immunochromatography (BinaxNOW)	15 minutes	Influenza A: 44% Influenza B: 25% RSV: 63 – 65%	Influenza A: 100% Influenza B: 100% RSV: 100%
Fluorescent immunoassay (Sofia)	15 minutes	Influenza A: 71.4% Influenza B: 33.3% RSV: 92.9%	Influenza A: 98.2% Influenza B: 99.5% RSV: 100%
Loop mediated isothermal amplification (Alere i)	15 minutes	Influenza A: 77.8% Influenza B: 75%	Influenza A: 100% Influenza B: 99%
Photon fluorescent excitation (mariPOC)	20 minutes	Influenza A: 71% Influenza B: 86% RSV: 89%	Influenza A: 100% Influenza B: 98% RSV: 100%

Hazelton J Med Virol 2014, IORV 2015; Ivaska J Clin Virol 2013

PPV and NPV of RIDT depends on prevalence of flu

If flu prevalence is…	and specificity is…	then PPV is…
very low (2.5%)	good (98%)	poor (39 – 56%)
moderate (20%)	good (98%)	good (86 - 93%)

If flu prevalence is…	and sensitivity is…	then NPV is…
moderate (20%)	poor (50%)	moderate (86 – 89%)
high (40%)	poor (50%)	very good (93 – 94%)

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Molecular platforms with point-of-care testing potential

Alere™ i

- **FDA approved and CE marked isothermal nucleic acid amplification-based system that uses a fluorescence-based molecular signal.**
- **Results are generated within 15 min, with around 2 min of "hands on" time.**
- **Specifically designed to be used by non-laboratory clinical staff in an acute care environment and it is the only molecular platform that is FDA approved specifically as a POCT.**
  - **the sensitivity and specificity of the Alere I Influenza A&B assay was 99.3% and 98.1% for influenza A, and 97.6% and 100% for influenza B compared to viral culture and PCR**

FilmArray Respiratory Panel

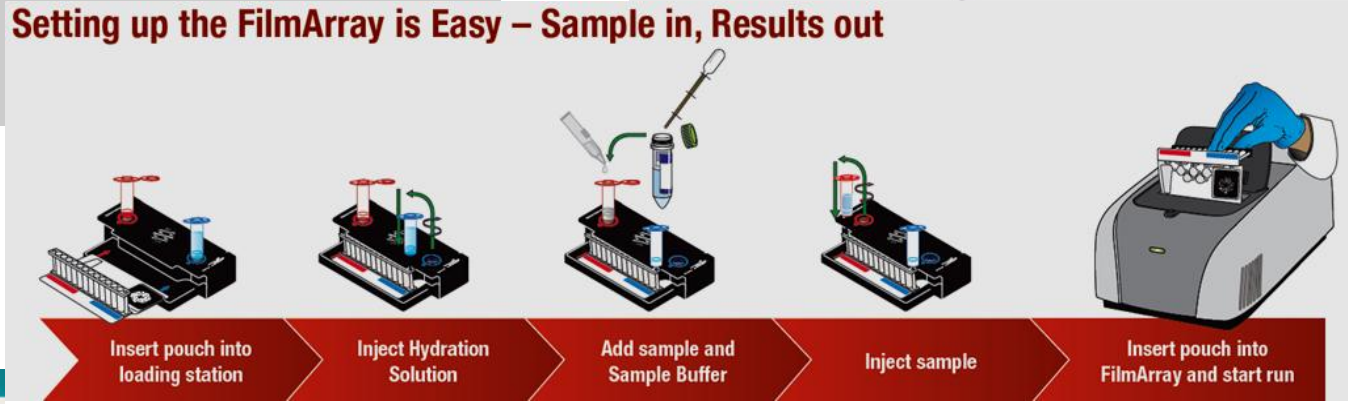
- nested real-time PCR to detect 20 respiratory pathogens
  - 17 viral targets and 3 bacteria
- 2 min of “hands on” time and produces a test result in one hour
- not used as a POCT but was housed within the existing laboratory
  - randomized controlled trials needed to date examining the potential clinical benefits of using this system as a POCT

Viruses

- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- Human Metapneumovirus
- Human Rhinovirus/ Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H1-2009
- Influenza A/H3
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus

Bacteria

- *Bordetella pertussis*
- *Chlamydophila pneumoniae*
- *Mycoplasma pneumoniae*



RSV POCTs

- Sensitivity
  - Good sensitivity in children during the RSV season
  - Lower sensitivity in adults with RSV infection
    - due to the lower and shorter period of RSV shedding
- Advantages
  - may assist with infection control, patient isolation, and cohorting, thus reducing nosocomial transmission
  - associated with reductions in antibiotic use, hospital costs, and length of stay
    - Mackie et al. reported a decrease in nosocomial RSV transmission
  - may also enable early institution of antiviral therapies in high-risk patients

Test	Manufacturer	Specimen	Sensitivity (%)	Specificity (%)	Time (min)
BinaxNOW® RSV	Binax, Scarborough, ME, USA	NPA, NPS, NW	70–93	89–100	15
Clearview® RSV	Inverness Medical Innovations, Bedford, UK	NPA[a], NPS, NW	93	97	15
Directigen RSV	Becton-Dickinson, Cockeysville, MD, USA	NPA, NPS, NW, tracheal aspirates	93–97	90–97	15
Directigen EZ RSV	Becton-Dickinson, Cockeysville, MD, USA	NPA, NPS, NW	89	93	15
Respi-Strip RSV	Coris Bio-Concept, Gembloux, Belgium	NPA, NPS	86–92	93–98	10
SAS™ RSV Alert	SA Scientific, San Antonio, TX, USA	NPA[a], nasal swab	83	91	10
Sure-Vue RSV	Thermo Fisher Scientific, Waltham, MA, USA	NPA[a], NPS, NW	96	94	15
Xpect RSV	Remel Inc, Lenexa, KS, USA	NPA[a], NPS	96	94	15

Conclusions

POCTs may be useful in a number of clinical situations

- For individual patient management (accepting the limitations of the assay and in conjunction with other laboratory tests, as required)
- In non-laboratory environments where trained staff are available to perform the assays
- During peak seasonal activity: e.g. influenza, RSV
- For diagnosis of outbreaks where the reduced sensitivity of the test may be overcome by testing multiple samples
- During periods when laboratory facilities are stretched: e.g. peak of seasonal influenza and during large outbreaks
- In laboratories with limited diagnostic facilities, although quality assurance, training and cost need to be considered in resource poor settings (33)
- Where early treatment is required: e.g. antivirals in influenza
- As a surveillance tool

Limitations of POCT

- POCTs often have reduced sensitivity compared to standard laboratory methods including nucleic acid testing (e.g. respiratory viruses)
- Subtyping of virus may not be available with POCTs (e.g. influenza)
- No isolate is available following POCTs for resistance testing or molecular epidemiology
- A second swab may be required for other tests (e.g. culture, PCR)
- Expense (especially if sequential testing)
- Samples are often collected by less experienced operators

Thank you for your patience ☺