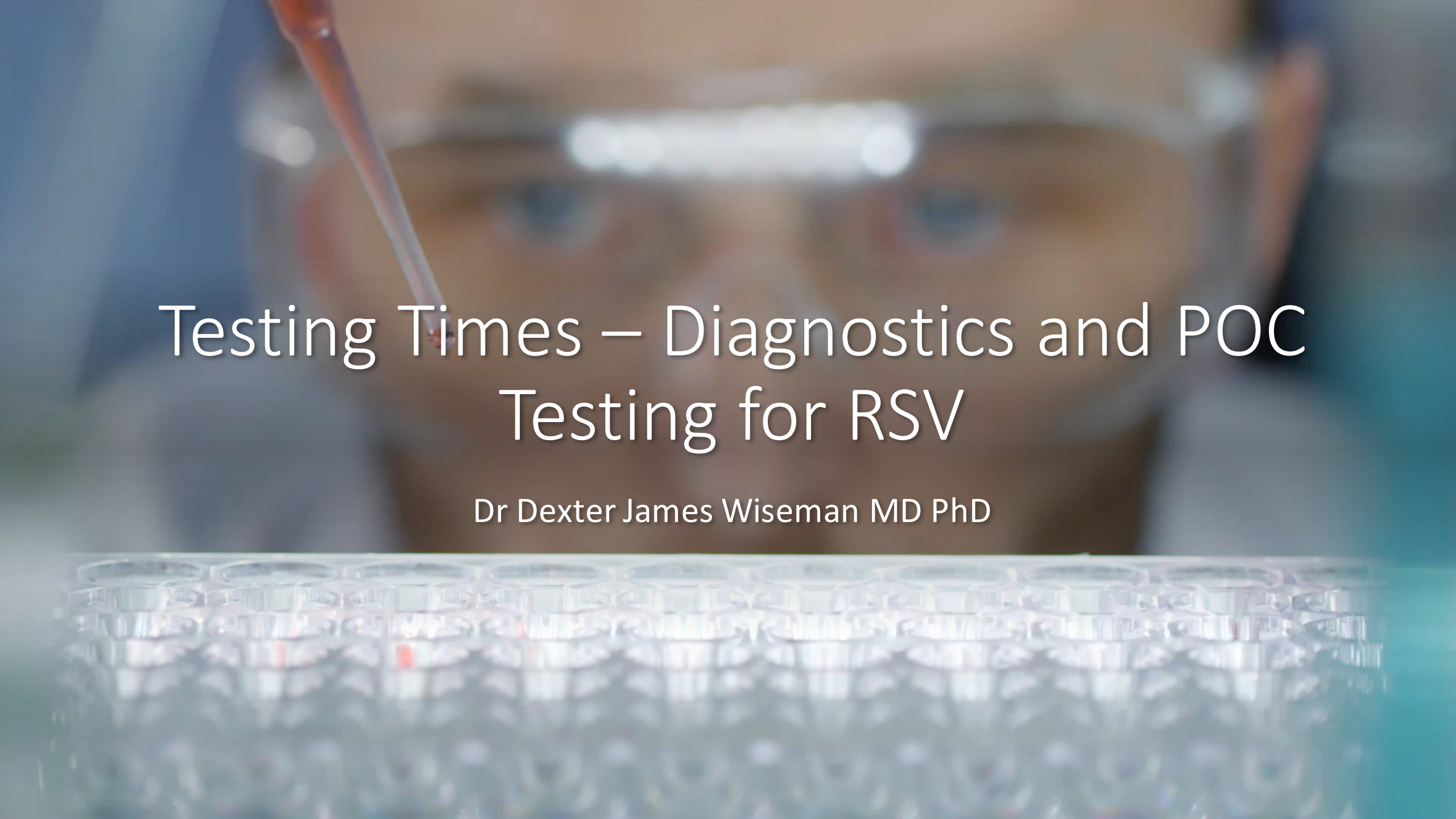


Testing Times – Diagnostics and POC Testing for RSV

Dr Dexter James Wiseman MD PhD

Conflicts of Interest

- I have received honoraria from GSK, Sanofi, Pfizer, CSL and Janssen for taking part in advisory boards and expert meetings and for acting as a speaker in congresses outside the scope of the work discussed.

Talk Outline

- Methodologies Available for RSV Diagnostics
- RSV Symptoms
- RSV Testing in Infants
- RSV Testing in Adults
- Awareness of RSV

Introduction: Testing Methodologies for RSV

Testing Methodologies for RSV

Viral Culture (historically gold standard):

Used Hep-2 / Human Fibroblasts and others

35-80% sensitive

Took 3-9 days for results

Less used since PCR

Limitations: Loss of cells, time to detection, issues with the cell lines, not practical in the clinical setting…

Total RSV testing for all subjects

Method or diagnosis	No. positive	No. tested	%
Culture	47	1,134	4.1
PCR	102	1,135	9.0
Serology	138	1,114	12.4
RSV by any method	166	1,495	11.1

Results of RSV diagnostic testing for subjects with all three tests available (n = 1,112)

No. of subjects	Culture	PCR	Serology
37	+	+	+
6	+	+	–
37	–	+	+
30	–	–	+
7[a]	–	+	–
995	–	–	–
Total no. of positives	43	87	104

[a]Defined as false-positive PCR results.

Hughes JH, Mann DR, Hamparian VV. Detection of respiratory syncytial virus in clinical specimens by viral culture, direct and indirect immunofluorescence, and enzyme immunoassay. J Clin Microbiol. 1988 Mar;26(3):588-91. doi: 10.1128/jcm.26.3.588-591.1988. PMID: 3281981; PMCID: PMC266341.

Falsey AR, Formica MA, Walsh EE. Diagnosis of respiratory syncytial virus infection: comparison of reverse transcription-PCR to viral culture and serology in adults with respiratory illness. J Clin Microbiol. 2002 Mar;40(3):817-20. doi: 10.1128/JCM.40.3.817-820.2002. PMID: 11880399; PMCID: PMC120281.

Testing Methodologies for RSV

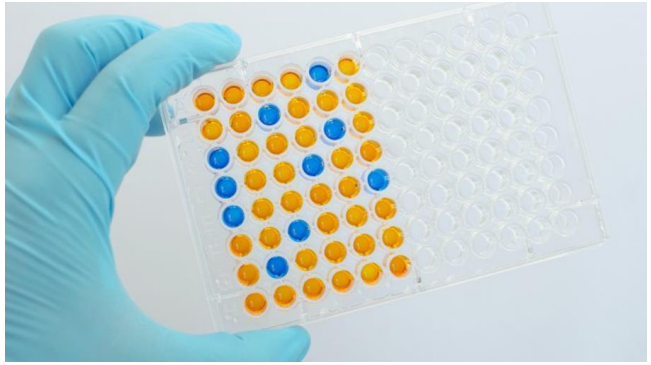
- Serology
- Usually based on Antibody testing

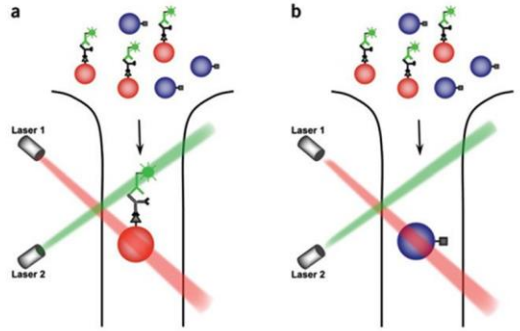
to RSV specific antigens

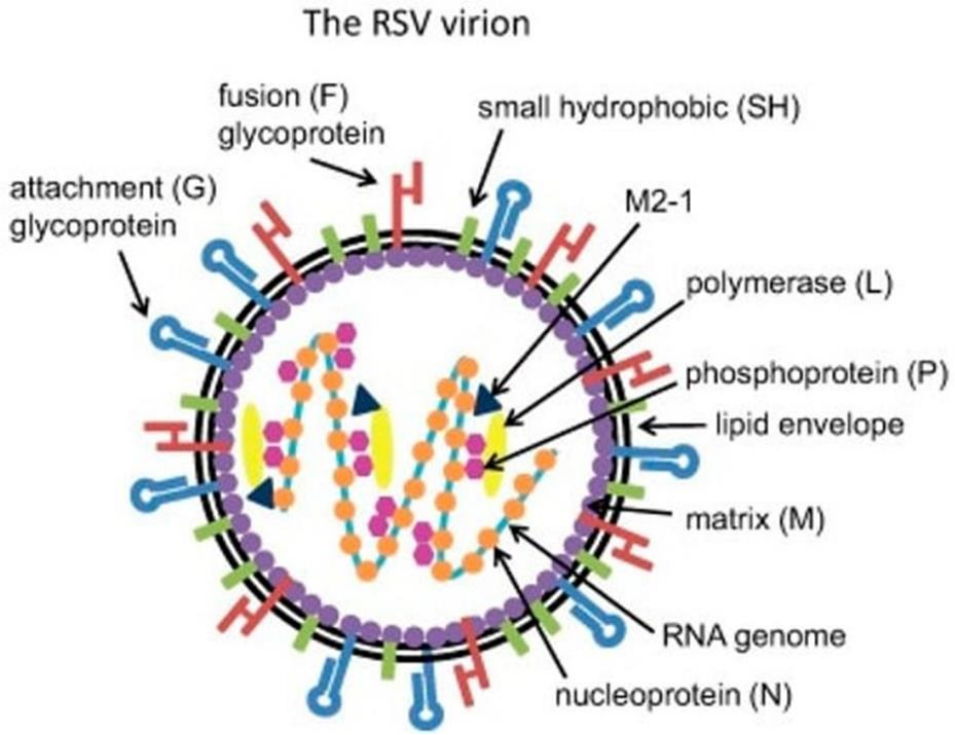
- 4-fold rise from baseline is considered

Positive (historical cut-off from ELISA)

- Antibodies to Pre/Post-F, Ga/b, N



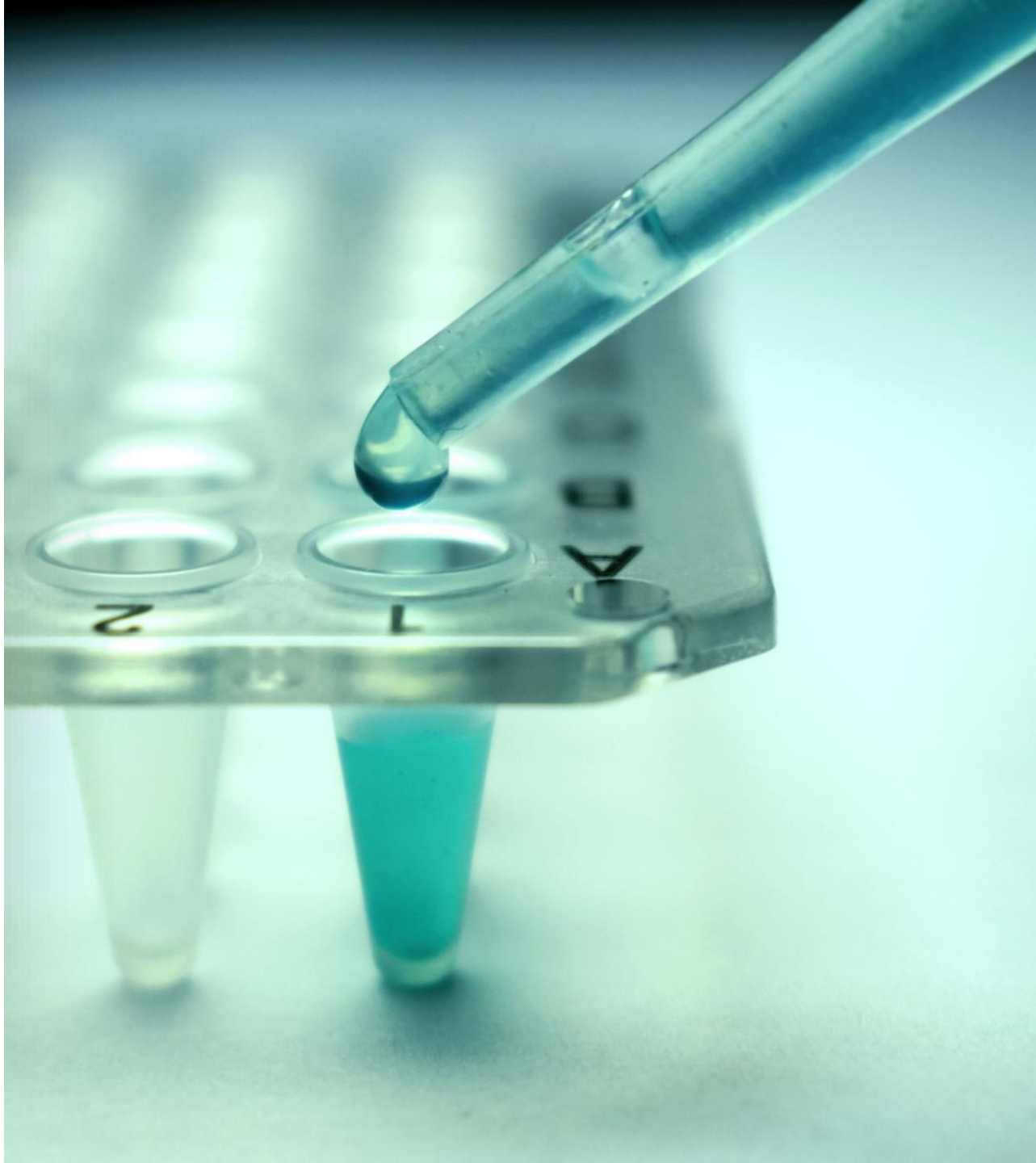




Adapted from Bianchini S et al. 2020[2]

Testing Methodologies for RSV

- PCR
- Can be specific for RSV A or B
- Multiplex available with other viruses (often a little less sensitive)
- Sequence selection important



Testing Methodologies for RSV

- Point of Care Testing
- Historically rapid-antigen style tests
- Now often PCR based
- Good accuracy when compared to standard PCR

	Standard testing strategy	POC testing strategy	Incremental difference (standard strategy – POC testing strategy)
POC informing use of isolation facilities			
Time to result (days)	1.24	0.15	1.09
Time in single room isolation (days)	1.62	1.15	0.47
Time in general bed (days)	0.28	0.75	−0.47
Cost of bed days	£1068400.00	£1020000.00	£48400.00
Cost of barrier nursing	£38310.00	£29700.00	£8610.00
Treatment costs	£440.00	£440.00	£0.00
Total costs	£1107150.00	£1050140.00	£57010.00

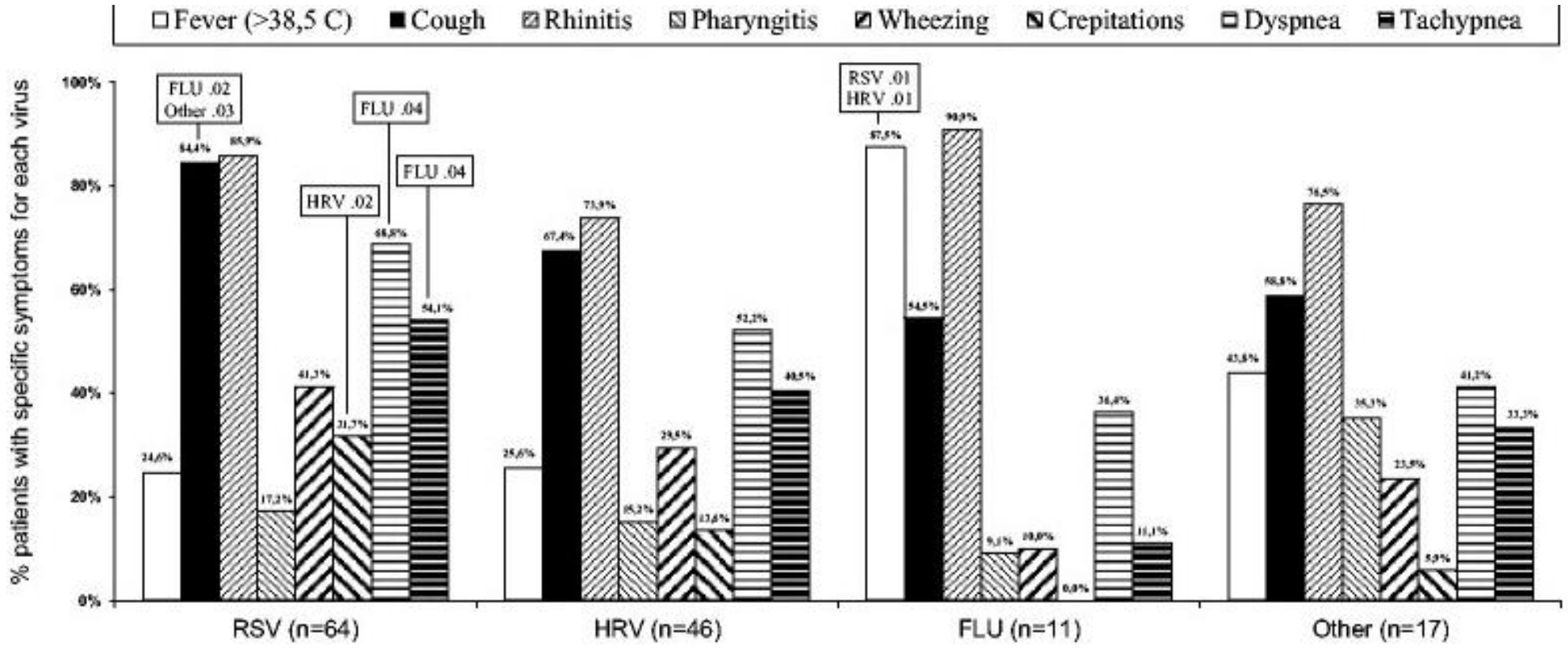
Diagnostic performance for respiratory syncytial virus (RSV) detection. Initial, or if necessary repeat, cobas® Liat® test, no discrepancy analysis

		Laboratory PCR RSV result		
		Positive	**Negative**	
cobas® Liat® RSV result	**Test positive**	90	4	94
	Test negative	1	66	67
		91	70	161

Diagnostic and economic evaluation of a point-of-care test for respiratory syncytial virus
A. Joy Allen, Andrea Gonzalez-Ciscar, Clare Lendrem, Jana Suklan, Karen Allen, Ashley Bell, Frances Baxter, Stephen Crulley, Louise Fairlie, Danielle Hardy, Louise Johnston, Joanne McKenna, Nicole Richards, Gavin Shovlin, Clare Simmister, Sheila Waugh, Philip Woodsford, Sara Graziadio, Michael Power, A. John Simpson, Prashant Kumar, Katherine Eastham, Malcolm Brodlie
ERJ Open Research Jul 2020, 6 (3) 00018-2020; **DOI:** 10.1183/23120541.00018-2020

Critical Issue:

It is not possible to diagnose RSV on symptoms alone:



Variation in symptoms in infants / children

- Gooskens, J., van der Ploeg, V., Sukhai, R.N. *et al.* Clinical evaluation of viral acute respiratory tract infections in children presenting to the emergency department of a tertiary referral hospital in the Netherlands. *BMC Pediatr* **14**, 297 (2014).

Symptoms of RSV infection compared to other common viral pathogens in older adults

Frequency of Symptoms Among Single Virus Infections (Viruses Detected in at Least 30 Cases)

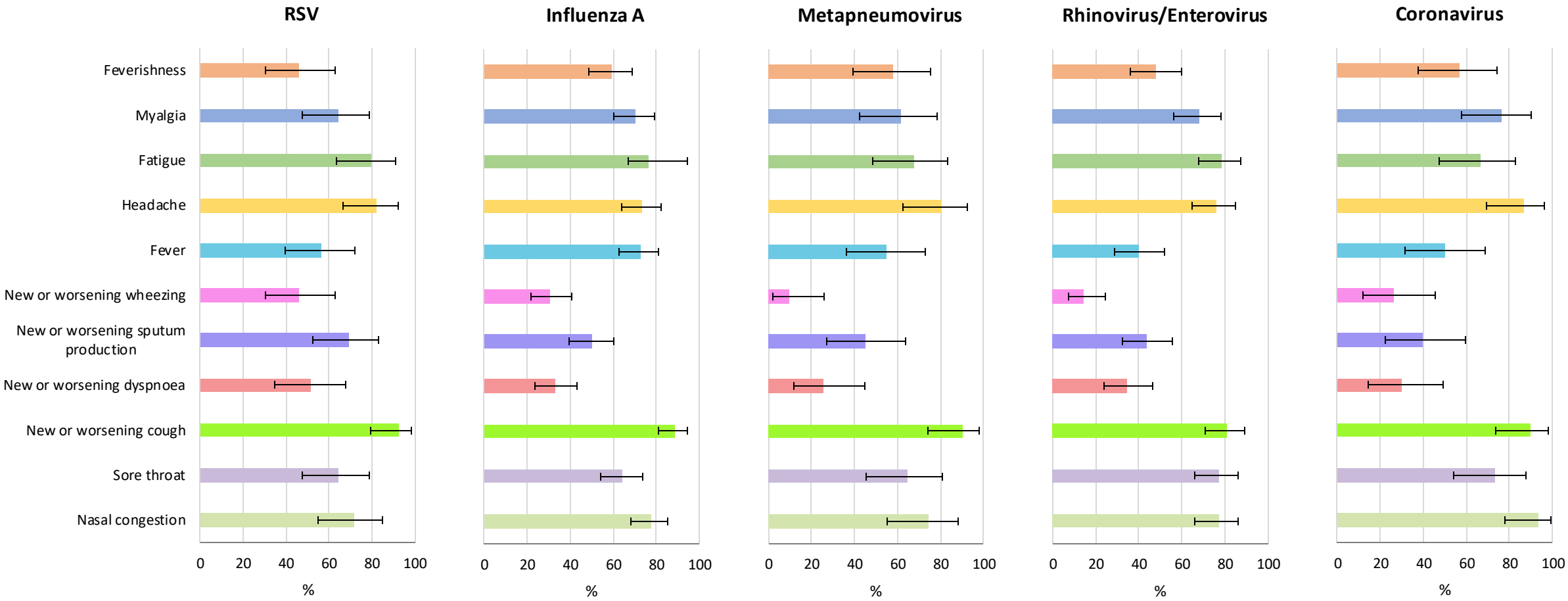
No. of Episodes (N)	RSV (N = 39)		Influenza A (N = 98)		Metapneumovirus (N = 31)		Rhinovirus/Enterovirus (N = 75)		Coronavirus (N = 30)	
Symptom	**n[a]**	**% (95% CI)**	**n**	**% (95% CI)**	**n**	**% (95% CI)**	**n**	**% (95% CI)**	**n**	**% (95% CI)**
Nasal congestion	28	71.8 (55.1–85.0)	76	77.6 (68.0–85.4)	23	74.2 (55.4–88.1)	58	77.3 (66.2–86.2)	28	93.3 (77.9–99.2)
Sore throat	25	64.1 (47.2–78.8)	63	64.3 (54.0–73.7)	20	64.5 (45.4–80.8)	58	77.3 (66.2–86.2)	22	73.3 (54.1–87.7)
New or worsening cough	36	92.3 (79.1–98.4)	87	88.8 (80.8–94.3)	28	90.3 (74.2–98.0)	61	81.3 (70.7–89.4)	27	90.0 (73.5–97.9)
New or worsening dyspnea	20	51.3 (34.8–67.6)	32	32.7 (23.5–42.9)	8	25.8 (11.9–44.6)	26	34.7 (24.0–46.5)	9	30.0 (14.7–49.4)
New or worsening sputum production	27	69.2 (52.4–83.0)	49	50.0 (39.7–60.3)	14	45.2 (27.3–64.0)	33	44.0 (32.5–55.9)	12	40.0 (22.7–59.4)
New or worsening wheezing	18	46.2 (30.1–62.8)	30	30.6 (21.7–40.7)	3	9.7 (2.0–25.8)	11	14.7 (7.6–24.7)	8	26.7 (12.3–45.9)
Fever	22	56.4 (39.6–72.2)	71	72.4 (62.5–81.0)	17	54.8 (36.0–72.7)	30	40.0 (28.9–52.0)	15	50.0 (31.3–68.7)
Headache	32	82.1 (66.5–92.5)	72	73.5 (63.6–81.9)	25	80.6 (62.5–92.5)	57	76.0 (64.7–85.1)	26	86.7 (69.3–96.2)
Fatigue	31	79.5 (63.5–90.7)	75	76.5 (66.9–84.5)	21	67.7 (48.6–83.3)	59	78.7 (67.7–87.3)	20	66.7 (47.2–82.7)
Myalgia	25	64.1 (47.2–78.8)	69	70.4 (60.3–79.2)	19	61.3 (42.2–78.2)	51	68.0 (56.2–78.3)	23	76.7 (57.7–90.1)
Feverishness	18	46.2 (30.1–62.8)	58	59.2 (48.8–69.0)	18	58.1 (39.1–75.5)	36	48.0 (36.3–59.8)	17	56.7 (37.4–74.5)

Abbreviations: CI, confidence interval; RSV, respiratory syncytial virus.

[a] n, number of subjects in a given category.

Falsey AR et al. J Infect Dis 2014;209:1873–81

Symptoms of RSV infection compared to other common viral pathogens in older adults[1]



RSV = respiratory syncytial virus.

1. Falsey AR et al. J Infect Dis. 2014;209(12):1873-1881.

RSV is indistinguishable from Flu in community based on symptoms:

Clinical symptoms of respiratory episodes

Patient reported symptoms[#]	RSV ARTI episodes (n=36)	Influenza ARTI episodes (n=57)	Other ARTI episodes[¶] (n=657)
Rhinitis	36 (100)	55 (96)	624 (95)
Cough	35 (97)	55 (96)	572 (87)
Wheeze	16 (44)	26 (46)	223 (34)
Phlegm	34 (94)	52 (91)	466 (71)[+]
Dyspneoa	24 (67)	42 (74)	309 (47)[§]
Fever ≥38 °C	2 (6)	11 (19)	26 (4)
Feeling feverish	12 (33)	37 (65)[+]	191 (29)
Headache	27 (75)	45 (79)	348 (53)[§]
Myalgia	19 (53)	41 (72)	263 (40)
Disturbed sleep	26 (72)	51 (89)[§]	440 (67)
Feeling unwell	33 (91)	56 (98)	499 (76)[§]
Disturbance in daily activity	27 (75)	51 (89)	348 (53)[+]
Vital signs from home visit[##]			
Fever ≥38 °C	2 (6)	9 (16)	13 (2)
Respiratory rate >20 breaths·min^{-1}	6 (17)	8 (14)	63 (10)
S_{pO_2} <95%	5 (14)	10 (18)	39 (6)

Data are presented as n (%). Numbers represent respiratory episodes unless stated otherwise and statistical significance is compared to RSV ARTI episodes. RSV: respiratory syncytial virus; ARTI: acute respiratory tract infection; S_{pO_2}: oxygen saturation measured by pulse oximetry. [#]: at least once during the respiratory infection based on the symptom diary; [¶]: RSV and influenza negative infections based on PCR; [+]: p<0.01; [§]: p<0.05; [f]: p<0.001 (not indicated if non-significant); [##]: measured by the study team.

	Healthy, Age ≥ 65 y		High Risk*, Age ≥ 21 y†	
Symptom	**RSV (%) n = 48**	**Influenza A (%) n = 18**	**RSV (%) n = 54**	**Influenza A (%) n = 16**
Nasal congestion	83	83	65	79
Cough	79	83	78	87
Sputum production	64	61	66	80
Dyspnea	9	28	58	71
Wheeze	23	17	50	50
Constitutional	53	72	59	71
Fever	18	44	31	47

Table compiled by E.E.W., with data from Falsey et al (*N Engl J Med*. 2005;352:1749–1759).[2]
*High risk defined as having physician-diagnosed CHF or chronic pulmonary disease.
†10% aged younger than 54, 17% aged 55 to 64, and 73% aged 65 years and older.

Symptoms in Outpatients with Laboratory-Confirmed RSV Versus Influenza A Through 4 Seasons, 1999–2003—Rochester, NY

Korsten K, Adriaenssens N, Coenen S, Butler C, Ravanfar B, Rutter H, Allen J, Falsey A, Pirçon JY, Gruselle O, Pavot V, Vernhes C, Balla-Jhagjhoorsingh S, Öner D, Ispas G, Aerssens J, Shinde V, Verheij T, Bont L, Wildenbeest J; RESCEU investigators. Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study. Eur Respir J. 2021 Apr 1;57(4):2002688. doi: 10.1183/13993003.02688-2020. PMID: 33060153.

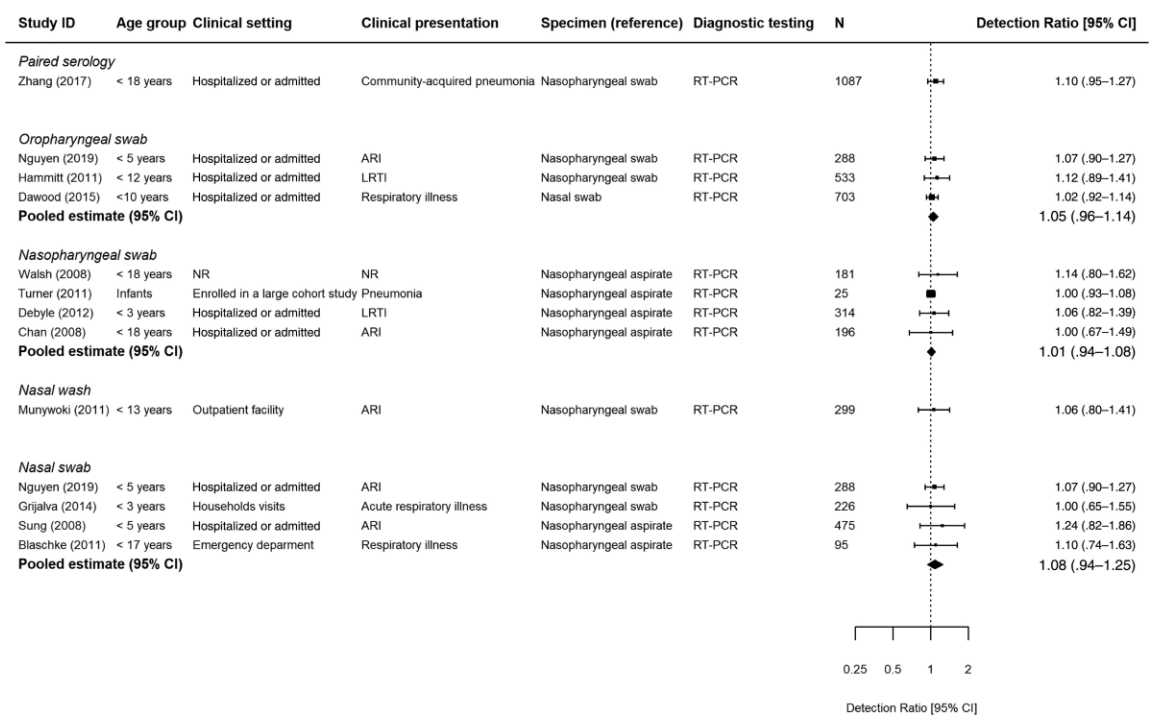
Talbot, H. Keipp MD, MPH[*]; Belongia, Edward A. MD[†]; Walsh, Edward E. MD[‡]; Schaffner, William MD[*]. Respiratory Syncytial Virus in Older Adults: A Hidden Annual Epidemic. Infectious Diseases in Clinical Practice 24(6):p 295-302, November 2016. | DOI: 10.1097/IPC.0000000000000455

RSV Testing in Infants

- More established than testing in adults
- Viral load is in infants is generally higher than in adults
- POC testing more sensitive

Testing in infants / children

- Recent meta-analysis of 157 papers
- RT-PCR was the most sensitive paediatric RSV diagnostic test. Adding multiple specimens did not substantially increase RSV detection, but even small proportional increases could result in meaningful changes in burden estimation.
- Adding paired serology testing increased RSV detection by 10%, Nasal Swab by 8%, oropharyngeal swabs by 5%, and NPS by 1%.
- Compared to RT-PCR, direct fluorescence antibody tests, viral culture, and rapid antigen tests were 87%, 76%, and 74% sensitive, respectively.
- Pooled sensitivity of multiplex versus singleplex RT-PCR was 96%

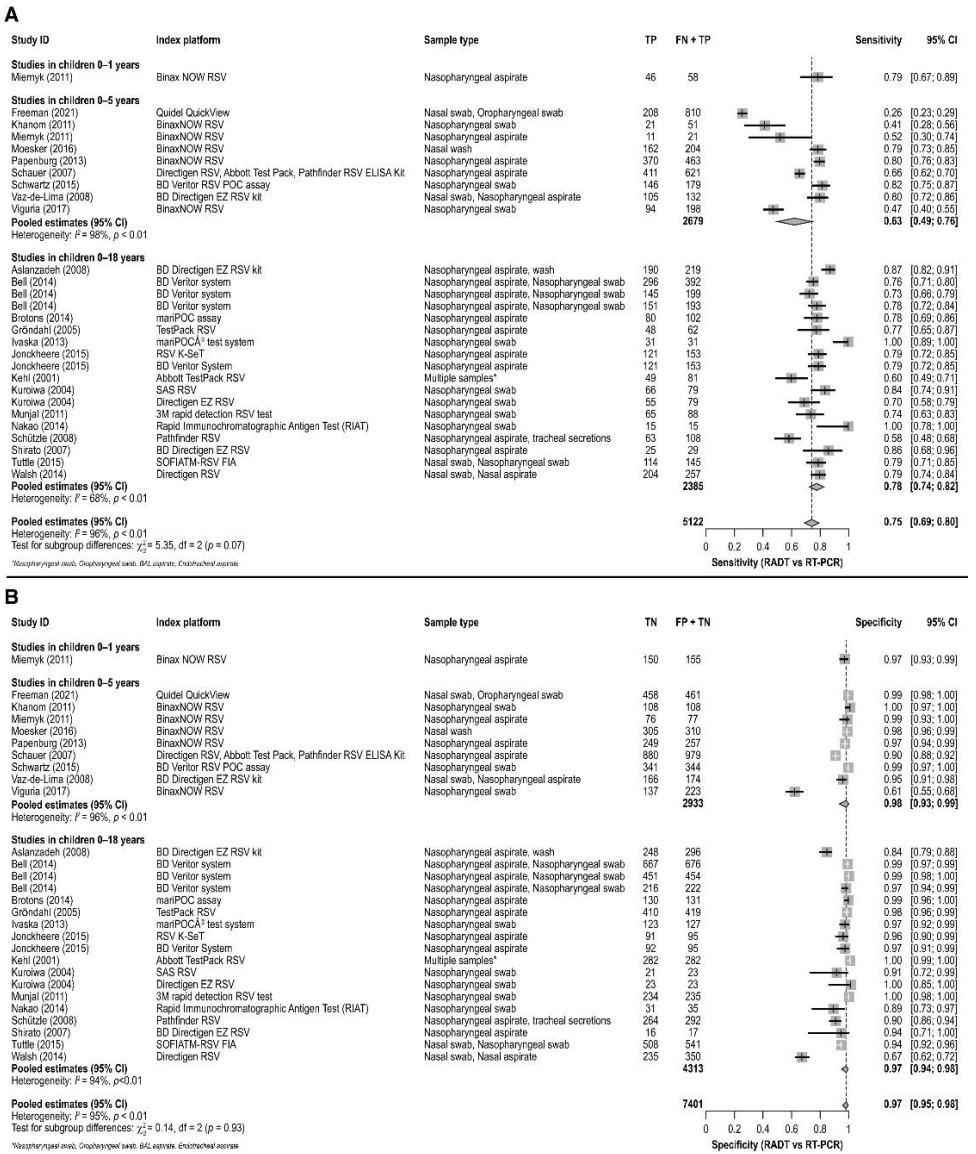


Study ID	Age group	Clinical setting	Clinical presentation	Specimen (reference)	Diagnostic testing	N	Detection Ratio [95% CI]
Paired serology							
Zhang (2017)	< 18 years	Hospitalized or admitted	Community-acquired pneumonia	Nasopharyngeal swab	RT-PCR	1087	1.10 (.95–1.27)
Oropharyngeal swab							
Nguyen (2019)	< 5 years	Hospitalized or admitted	ARI	Nasopharyngeal swab	RT-PCR	288	1.07 (.90–1.27)
Hammitt (2011)	< 12 years	Hospitalized or admitted	LRTI	Nasopharyngeal swab	RT-PCR	533	1.12 (.89–1.41)
Dawood (2015)	<10 years	Hospitalized or admitted	Respiratory illness	Nasal swab	RT-PCR	703	1.02 (.92–1.14)
Pooled estimate (95% CI)							1.05 (.96–1.14)
Nasopharyngeal swab							
Walsh (2008)	< 18 years	NR	NR	Nasopharyngeal aspirate	RT-PCR	181	1.14 (.80–1.62)
Turner (2011)	Infants	Enrolled in a large cohort study	Pneumonia	Nasopharyngeal aspirate	RT-PCR	25	1.00 (.93–1.08)
Debyle (2012)	< 3 years	Hospitalized or admitted	LRTI	Nasopharyngeal aspirate	RT-PCR	314	1.06 (.82–1.39)
Chan (2008)	< 18 years	Hospitalized or admitted	ARI	Nasopharyngeal aspirate	RT-PCR	196	1.00 (.67–1.49)
Pooled estimate (95% CI)							1.01 (.94–1.08)
Nasal wash							
Munywoki (2011)	< 13 years	Outpatient facility	ARI	Nasopharyngeal swab	RT-PCR	299	1.06 (.80–1.41)
Nasal swab							
Nguyen (2019)	< 5 years	Hospitalized or admitted	ARI	Nasopharyngeal swab	RT-PCR	288	1.07 (.90–1.27)
Grijalva (2014)	< 3 years	Households visits	Acute respiratory illness	Nasopharyngeal swab	RT-PCR	226	1.00 (.65–1.55)
Sung (2008)	< 5 years	Hospitalized or admitted	ARI	Nasopharyngeal aspirate	RT-PCR	475	1.24 (.82–1.86)
Blaschke (2011)	< 17 years	Emergency deparment	Respiratory illness	Nasopharyngeal aspirate	RT-PCR	95	1.10 (.74–1.63)
Pooled estimate (95% CI)							1.08 (.94–1.25)

Chukwuemeka Onwuchekwa, Jessica Atwell, Laura Mora Moreo, Sonia Menon, Belen Machado, MarianaSiapka, Neha Agarwal, Michelle Rubbrecht, Zuleika Aponte-Torres, Mark Rozenbaum, Daniel Curcio, Harish Nair, Warren V Kalina, Hilde Vroling, Bradford Gessner, Elizabeth Begier, Pediatric Respiratory Syncytial Virus Diagnostic Testing Performance: A Systematic Review and Meta-analysis, *The Journal of Infectious Diseases*, Volume 228, Issue 11, 1 December 2023, Pages 1516–1527, https://doi.org/10.1093/infdis/jiad185

POC vs RT-PCR in infants

Sensitivity varied from 26-100% comparing monoplex PCR to POCT



J Infect Dis, Volume 228, Issue 11, 1 December 2023, Pages 1516–1527, https://doi.org/10.1093/infdis/jiad185

Multiplex PCR is less sensitive than monoplex in infants

Virus	Concentration [copies/ml]	RVP				RespiFinder-19				RespiFinder- SMART-22				Monoplex real-time PCR			
		und.	1:10	1:100	1:1000	und.	1:10	1:100	1:1000	und.	1:10	1:100	1:1000	und.	1:10	1:100	1:1000
INF-A H1	1.78E+04	+	+	+	-	-	-	-	-	-	-	-	-	32.9	33.8	35.8	37.9
INF-A H3	1.42E+04	+	+	+	-	-	-	-	-	-	-	-	-				
INF-B	n. q.	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
RSV-A	3.94E+04	+	+	+	-	-	-	-	-	-	-	-	-	28.7	30.4	32.5	34.6
RSV-B	n. q.	+	+	+	-	-	-	-	-	-	-	-	-	x	x	x	x
PIV-1	1.61E+04	-	-	-	-	-	-	-	-	-	-	-	-	32.8	33.1	34.9	36.6
PIV-2	9.19E+03	-	-	-	-	+	-	-	-	+	+	+	-	29.5	31.4	33.7	36.4
PIV-3	3.84E+04	-	-	-	-	-	-	-	-	-	-	-	-	34.9	36.8	38.3	39.8
CoV OC43	n. q.	+	+	+	+	+	+	+	+	+	+	+	+	27.6	28.9	30.1	31.9
CoV 229E	n. q.	-	-	-	-	+	+	+	+	+	+	+	+	26.8	27.9	29.1	30.5
HRV	2.83E+04	+	+	+	+	+	+	-	-	+	+	+	-	31.2	33.8	36.0	38.4
AdV	3.56E+04	-	-	-	-	+	+	+	+	+	+	+	-	35.9	37.6	38.8	39.9
hMPV	1.78E+04	+	+	+	+	-	-	-	-	-	-	-	-	30.2	32.0	34.8	38.4

n. q.: not quantified; x: not performed; und.: undiluted; The dilution series were done as two-fold dilution series.

Table 3. Sensitivity of two kinds of ResPlex II kit for common viruses *

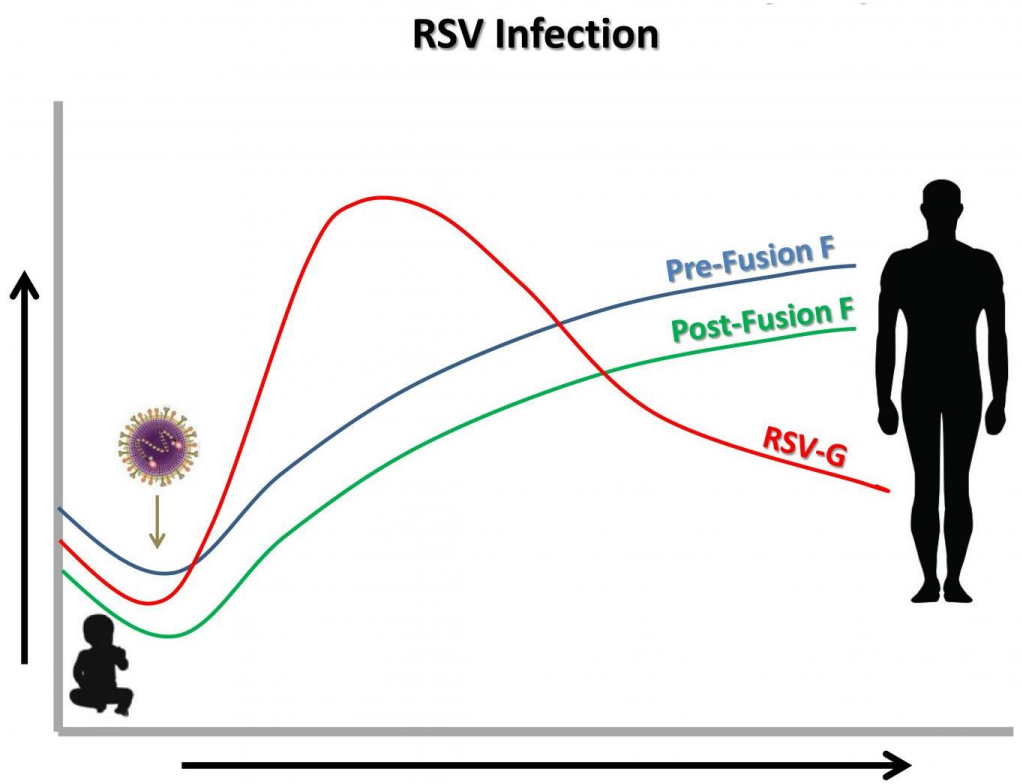
Virus	Resplex II Panel No. of specimens				Sensitivity (%)	ResPlex II Plus Panel PRE No. of specimens				Sensitivity (%)	χ^2 value	*P* value
	M+R+	M+R-	M-R+	M-R-		M+R+	M+R-	M-R+	M-R-			
RSV	14	25	3	82	35.9	32	67	3	212	32.3	0.161	0.688
FluA	2	2	2	118	50.0	2	30	0	282	6.3	6.891	0.053
hMPV	4	4	2	114	50.0	17	5	1	291	77.3	2.078	0.195
PIV3	6	0	2	116	100.0	13	7	3	291	65.0	2.874	0.146
PIV1	3	0	2	119	100.0	9	6	1	298	60.0	1.80	0.515
hBoV	5	1	0	118	83.3	5	7	0	302	41.7	2.813	0.152

* R, ResPlex II Panel; M, monoplex real-time TaqMan RT-PCR.

- Deng, J., Ma, Z., Huang, W. *et al.* Respiratory virus multiplex RT-PCR assay sensitivities and influence factors in hospitalized children with lower respiratory tract infections. *Virol. Sin.* **28**, 97–102 (2013). https://doi.org/10.1007/s12250-013-3312-y
- Dabisch-Ruthe, M., Vollmer, T., Adams, O. *et al.* Comparison of three multiplex PCR assays for the detection of respiratory viral infections: evaluation of xTAG respiratory virus panel fast assay, RespiFinder 19 assay and RespiFinder SMART 22 assay. *BMC Infect Dis* **12**, 163 (2012). https://doi.org/10.1186/1471-2334-12-163

Serology in Infants

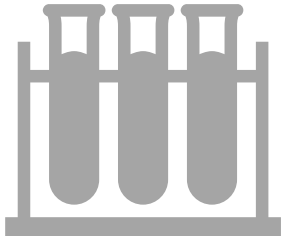
- If first infection, minimal RSV specific antibodies in serum
- Some antibodies detectable in early infancy from maternal transfer
- All children will have been infected by ~ age of 2
- Evidence of maternal antibodies affording protection



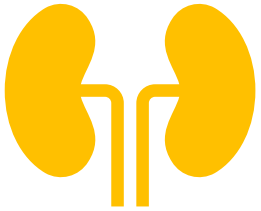
Andrea G Buchwald, Barney S Graham, Awa Traore, Fadima Cheick Haidara, Man Chen, Kaitlyn Morabito, Bob C Lin, Samba O Sow, Myron M Levine, Marcela F Pasetti, Milagritos D Tapia, Respiratory Syncytial Virus (RSV) Neutralizing Antibodies at Birth Predict Protection from RSV Illness in Infants in the First 3 Months of Life, *Clinical Infectious Diseases*, Volume 73, Issue 11, 1 December 2021, Pages e4421–e4427, https://doi.org/10.1093/cid/ciaa648

Serology Sensitivity in Infants

Generally, does not add substantially to PCR



Lysate + F-based antibody tests up to 100% sensitive



Difficult in practice as pre-illness serum needed (some studies use cord blood for comparison).

Anderson LJ, Jadhao SJ, Hussaini L, Ha B, McCracken CE, Gibson T, Yildirim I, Yi J, Stephens K, Korski C, Kao C, Sun H, Lee CY, Jaunarajs A, Rostad CA, Anderson EJ. Development and comparison of immunologic assays to detect primary RSV infections in infants. Front Immunol. 2024 Jan 12;14:1332772. doi: 10.3389/fimmu.2023.1332772. PMID: 38283339; PMCID: PMC10811012.

Testing in Adults

- As we have heard there is significant burden of RSV amongst older adults
- There is an issue of clinical governance with regards to diagnostics
- Accurate testing will help inform vaccine roll-out and decision makers

Diagnostic barriers for RSV in Older Adults:

Clinical diagnosis is not possible due to similarities with other viral diseases

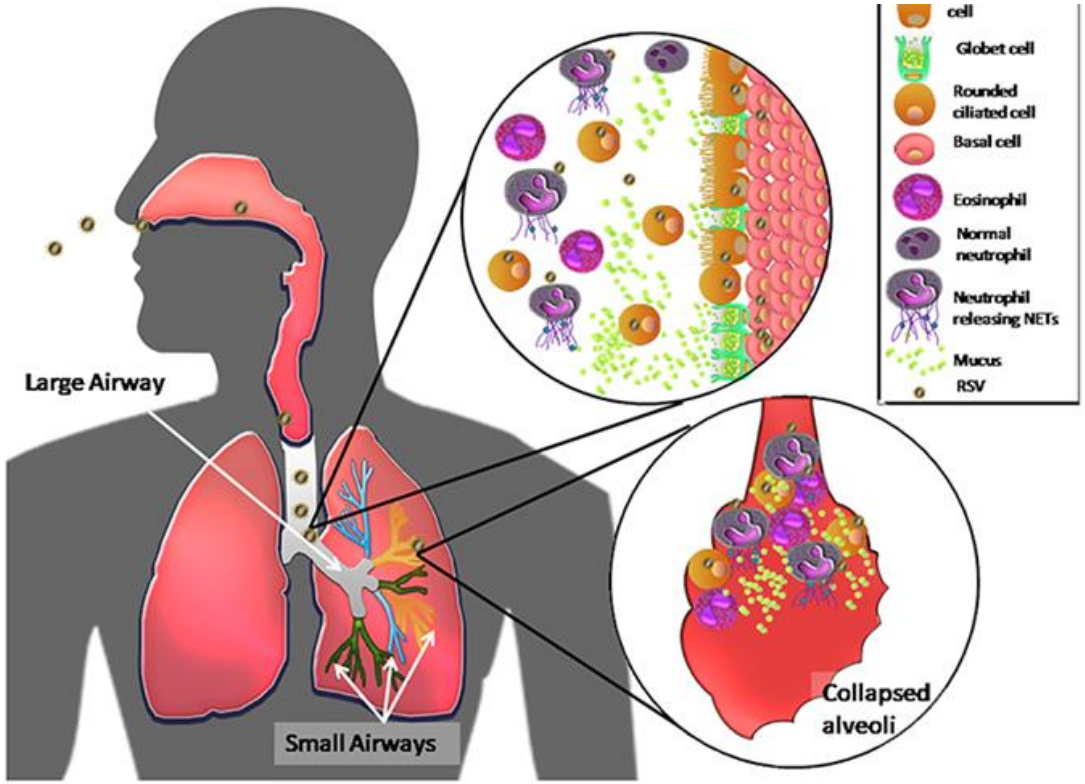
Lack of incentive to diagnose RSV as no dedicated treatment exists

Many cases, especially in primary care, currently go undiagnosed

Relatively high cost of polymerase chain reaction analysis (developing countries specifically)

Practical Issues with Diagnostics

- PCR based diagnostic techniques are however not 100% sensitive in adults.
- There is a propensity for RSV to replicate in the lower airways, therefore nasal swabbing alone might not be sufficient to obtain a diagnosis.
- Multiple methods often used in studies, not practical in the clinical setting
- Older POC testing was not sensitive in adults, newer systems however show promise
- Adults shed considerably less virus than infants ($\leq 10^3$ versus $\leq 10^6$ PFU/ml)



Adapted from Carvajal et al. 2019[2]

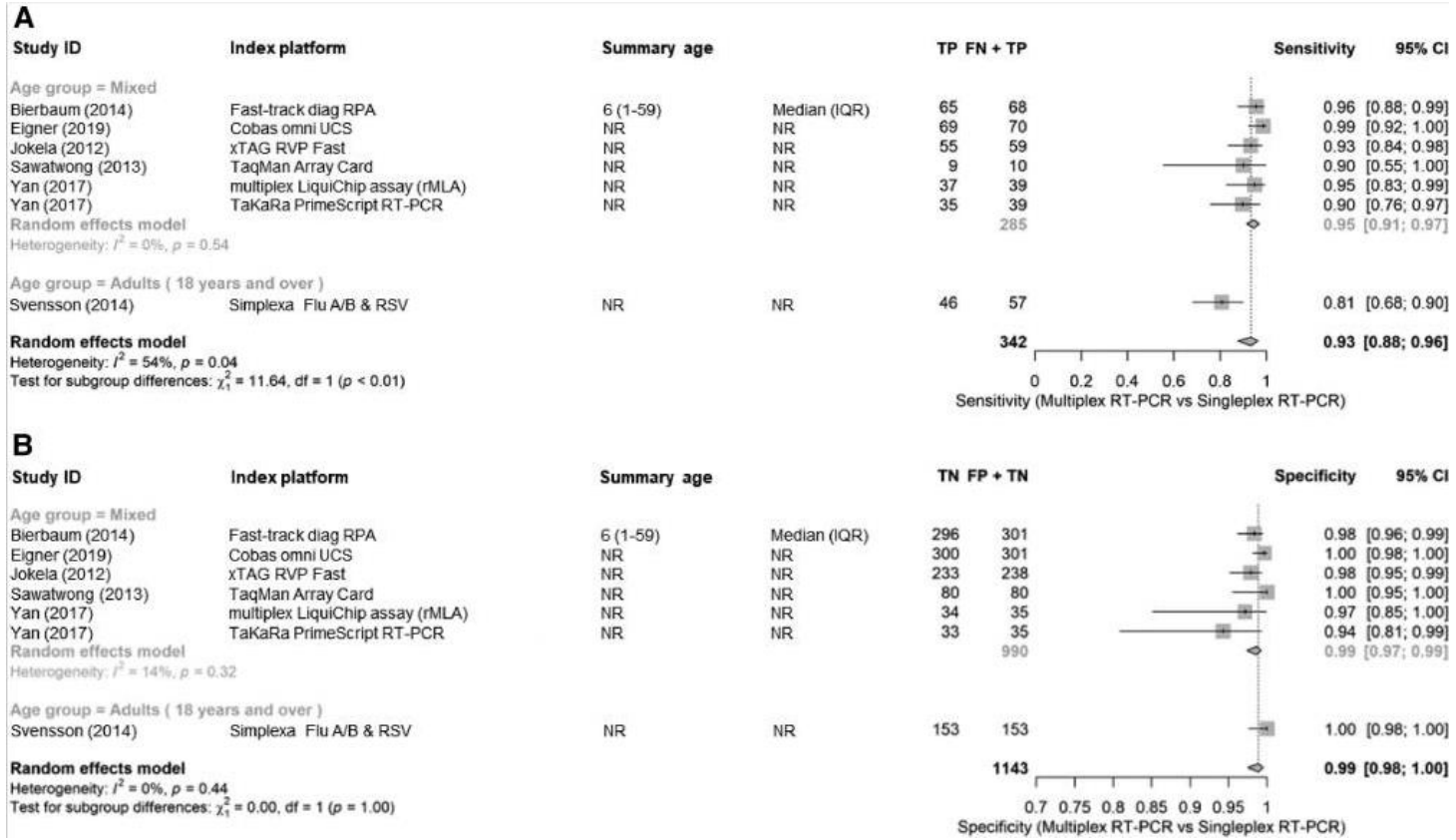
Viral Culture Adults

- 3 studies that assessed viral culture versus a reference standard of RT-PCR. The sensitivity ranged between 49% and 86%.
- Diagnosis of RSV infection by culture is considerably more difficult than diagnosis of influenza, with sensitivities ranging from 17% to 39%, compared with serological tests and PCR

Onwuchekwa C, Moreo LM, Menon S, Machado B, Curcio D, Kalina W, Atwell JE, Gessner BD, Siapka M, Agarwal N, Rubbrecht M, Nair H, Rozenbaum M, Aponte-Torres Z, Vroling H, Begier E. Underascertainment of Respiratory Syncytial Virus Infection in Adults Due to Diagnostic Testing Limitations: A Systematic Literature Review and Meta-analysis. J Infect Dis. 2023 Jul 14;228(2):173-184. doi: 10.1093/infdis/jiad012. PMID: 36661222; PMCID: PMC10345483.

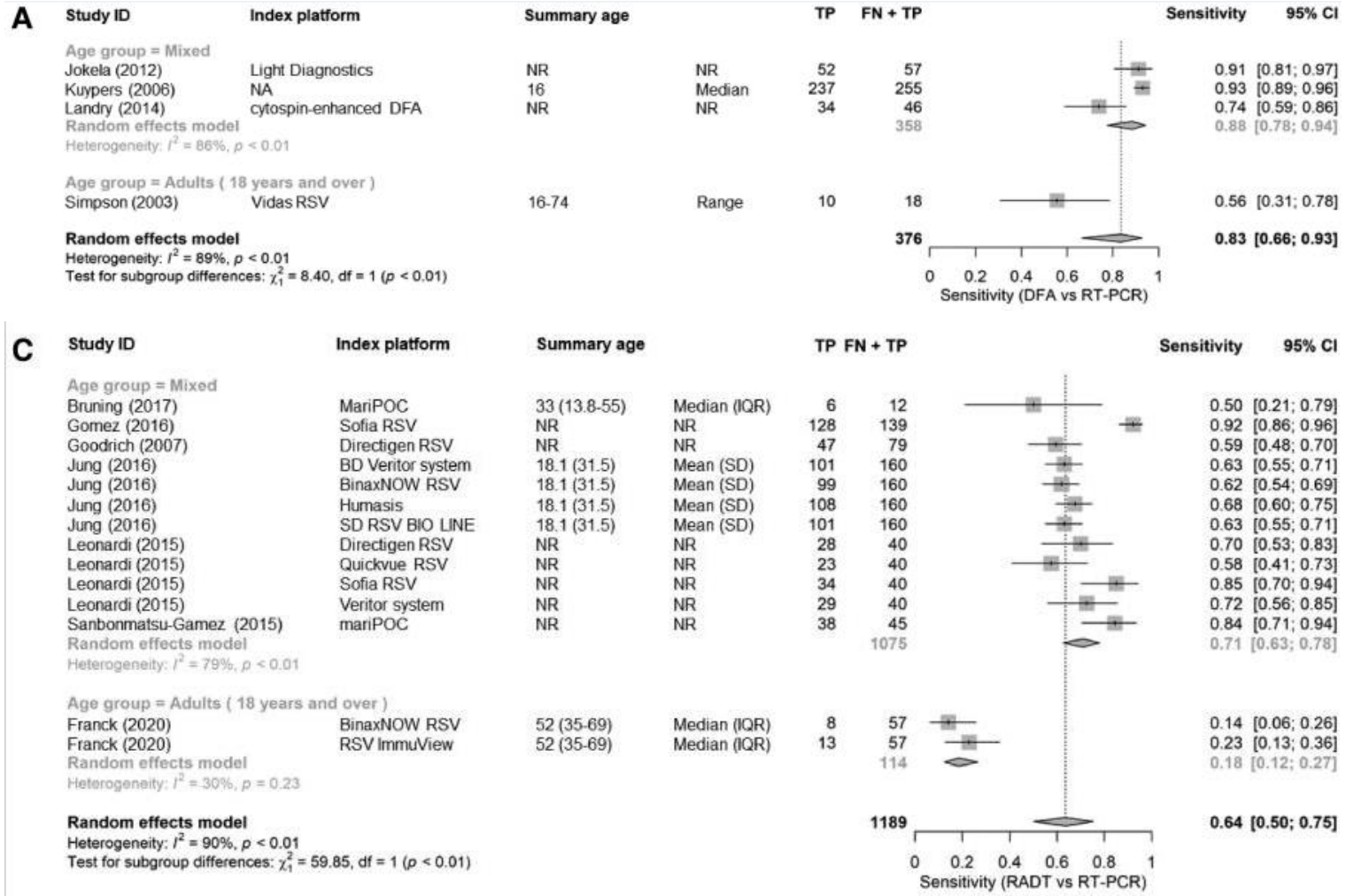
Multiplex is less sensitive than Monoplex in Adults

- Six studies evaluated the performance of a multiplex RT-PCR versus a monoplex platform. Pooled sensitivity was 92% and specificity 99%.



Onwuchekwa C, Moreo LM, Menon S, Machado B, Curcio D, Kalina W, Atwell JE, Gessner BD, Siapka M, Agarwal N, Rubbrecht M, Nair H, Rozenbaum M, Aponte-Torres Z, Vroling H, Begier E. Underascertainment of Respiratory Syncytial Virus Infection in Adults Due to Diagnostic Testing Limitations: A Systematic Literature Review and Meta-analysis. J Infect Dis. 2023 Jul 14;228(2):173-184. doi: 10.1093/infdis/jiad012. PMID: 36661222; PMCID: PMC10345483.

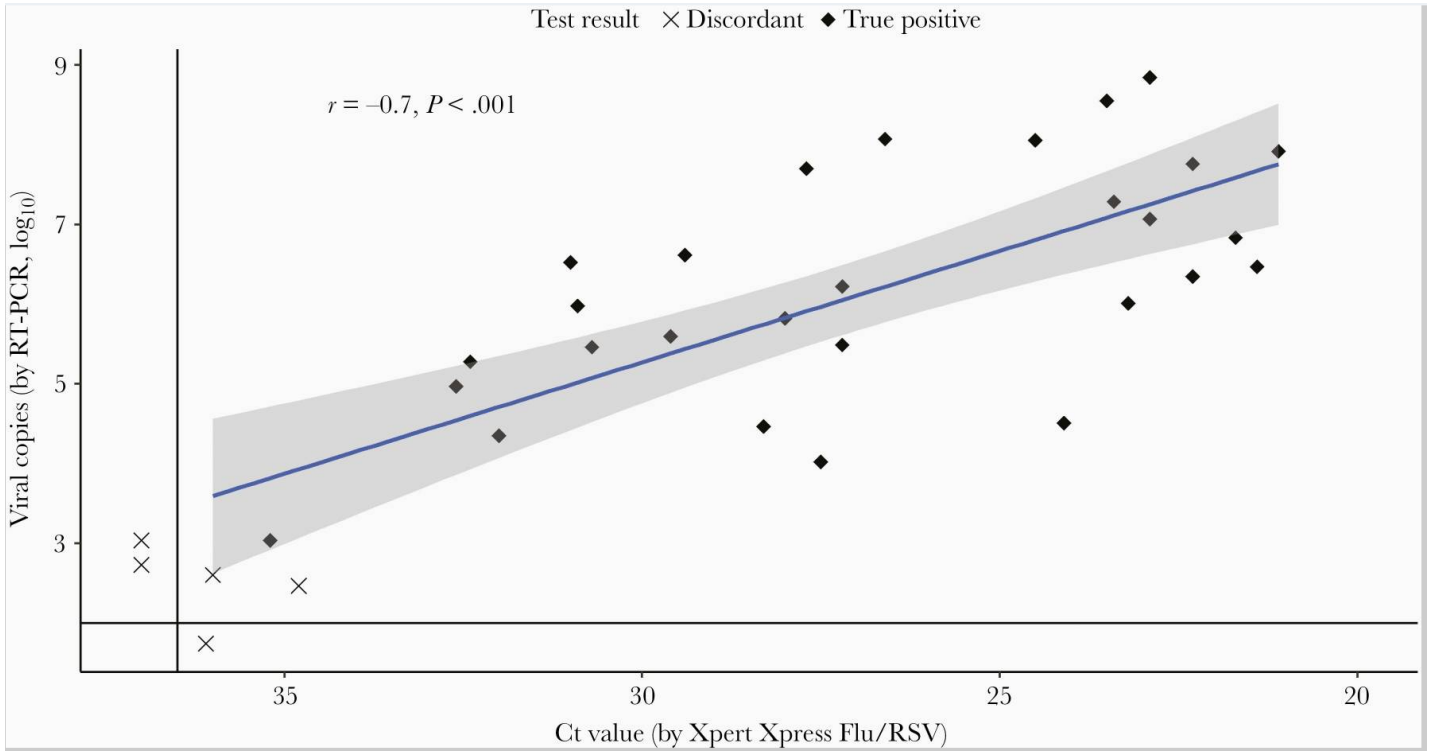
POCT vs PCR Adults

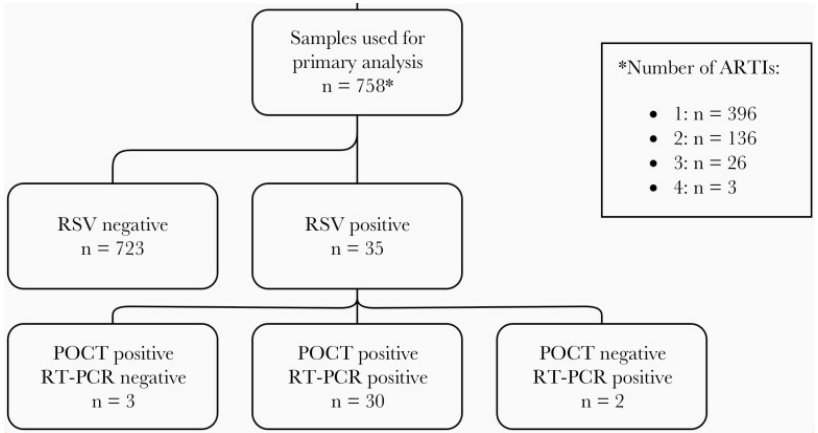


Onwuchekwa C, Moreo LM, Menon S, Machado B, Curcio D, Kalina W, Atwell JE, Gessner BD, Siapka M, Agarwal N, Rubbrecht M, Nair H, Rozenbaum M, Aponte-Torres Z, Vroling H, Begier E. Underascertainment of Respiratory Syncytial Virus Infection in Adults Due to Diagnostic Testing Limitations: A Systematic Literature Review and Meta-analysis. J Infect Dis. 2023 Jul 14;228(2):173-184. doi: 10.1093/infdis/jiad012. PMID: 36661222; PMCID: PMC10345483.

POC Testing

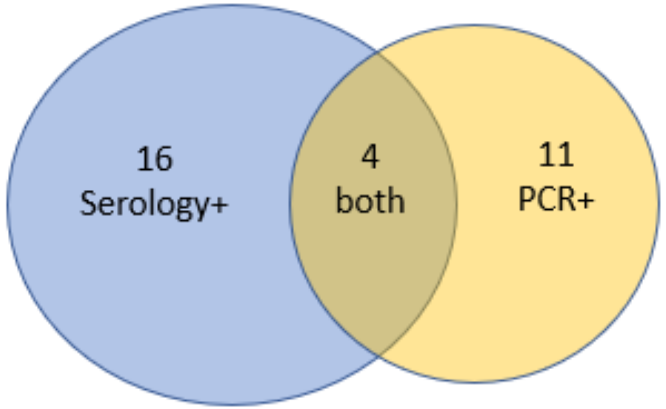
- POC Testing in Adults in the Outpatient setting





Zuurbier RP, Korsten K, Verheij TJM, Butler C, Adriaenssens N, Coenen S, Gruselle O, Vantomme V, van Houten MA, Bont LJ, Wildenbeest JG; REspiratory Syncytial Virus Consortium in EUrope (RESCEU) Investigators. Performance Assessment of a Rapid Molecular Respiratory Syncytial Virus Point-of-Care Test: A Prospective Community Study in Older Adults. J Infect Dis. 2022 Aug 12;226(Suppl 1):S63-S70. doi: 10.1093/infdis/jiab600. PMID: 35134954; PMCID: PMC9374508.

RSV Testing in Adults: Combining Techniques



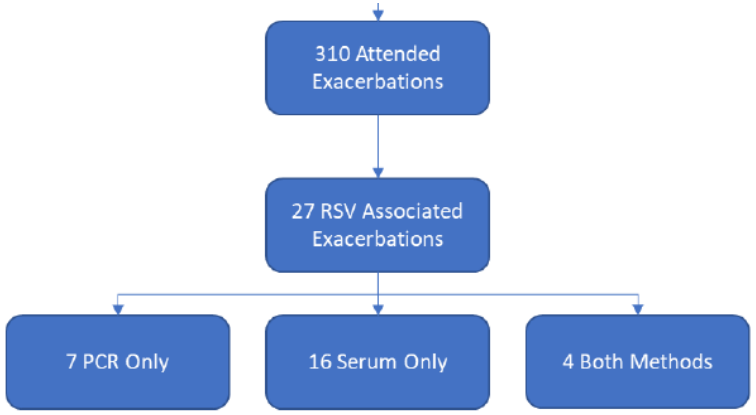


Table 2. RSV infection

	2017-2018 N = 527		2018-2019 N = 513	
	Cases	% (95% CI)	Cases	% (95% CI)
RSV-illness[a]	22	4.2% (2.6- 6.3)	37	7.2% (5.5 - 10.2)
PCR positive[b]	11	2.1% (1.0-3.7)	25	4.9% (3.2-7.1)
Seroconversion[c]	15	2.8% (1.6-4.7)	24	4.7% (3.0-6.9)

[a] Either positive PCR or evidence of seroconversion [b] Based on positive PCR or POCT [c] based on ≥4-fold increase in any antibody titer.

Wiseman, D et al Frequency of RSV-Related COPD Exacerbations Using Standard and Enhanced Diagnostic Methods: A Binational Prospective Cohort Study. Available at SSRN: https://ssrn.com/abstract=4487116 or http://dx.doi.org/10.2139/ssrn.4487116

Falsey AR, Walsh EE, Esser MT, Shoemaker K, Yu L, Griffin MP. Respiratory syncytial virus-associated illness in adults with advanced chronic obstructive pulmonary disease and/or congestive heart failure. J Med Virol. 2019 Jan;91(1):65-71. doi: 10.1002/jmv.25285. Epub 2018 Sep 24. PMID: 30132922; PMCID: PMC6900175.

Comparing PCR, saliva, sputum and serology

Prospective cohort study of patients aged ≥ 40 years hospitalized for acute respiratory illness (ARI)

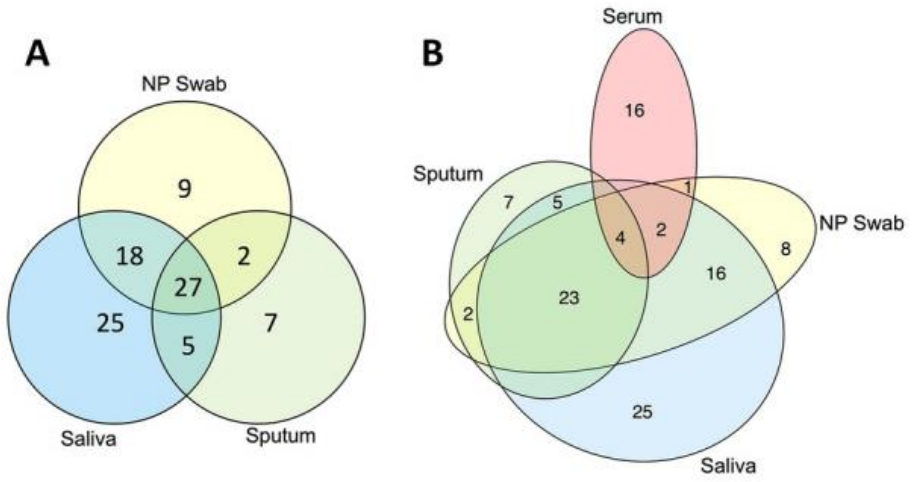
NP swab, saliva, and sputum specimens were collected at enrollment. Serology specimens were obtained at acute and convalescent timepoints.

NP swab plus additional specimens, corresponding to a 1.95 times higher rate.

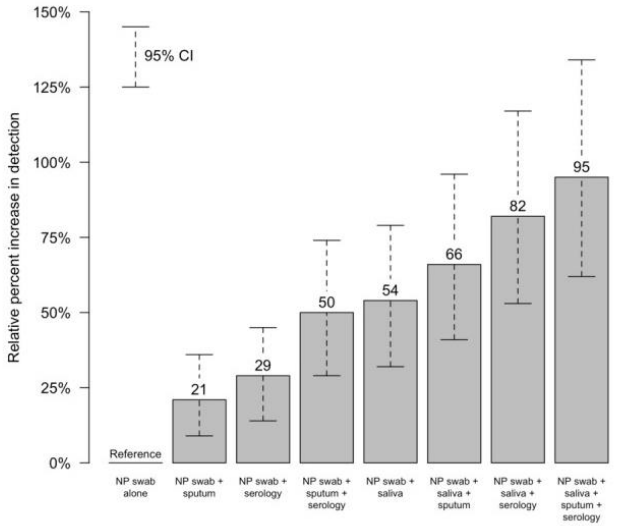
Sensitivities by specimen type were: NP swab 51%, saliva 70%, sputum 72%, and serology 79%.

Ramirez J, Carrico R, Wilde A, Junkins A, Furmanek S, Chandler T, Schulz P, Hubler R, Peyrani P, Liu Q, Trivedi S, Uppal S, Kalina WV, Falsey AR, Walsh EE, Yacisin K, Jodar L, Gessner BD, Begier E. Diagnosis of Respiratory Syncytial Virus in Adults Substantially Increases When Adding Sputum, Saliva, and Serology Testing to Nasopharyngeal Swab RT-PCR. Infect Dis Ther. 2023 Jun;12(6):1593-1603. doi: 10.1007/s40121-023-00805-1. Epub 2023 May 6. PMID: 37148463; PMCID: PMC10163290.

Comparing PCR, saliva, sputum and serology



Respiratory syncytial virus (RSV) diagnosis by specimen type: all specimen types contribute unique positives. **A** (left) Venn diagram of nasopharyngeal (NP) swab, saliva, and sputum specimens detecting RSV from RT–PCR diagnostic testing. **B** (right) Euler diagram of NP swab, saliva, sputum, and serology specimens diagnosing RSV



The percent increase in respiratory syncytial virus (RSV) diagnosis when adding additional specimen types in the analysis, over using nasopharyngeal (NP) specimens alone

Using all 4 specimens there was a 2.60-fold increase compared to NP swab alone

Ramirez J, Carrico R, Wilde A, Junkins A, Furmanek S, Chandler T, Schulz P, Hubler R, Peyrani P, Liu Q, Trivedi S, Uppal S, Kalina WV, Falsey AR, Walsh EE, Yacisin K, Jodar L, Gessner BD, Begier E. Diagnosis of Respiratory Syncytial Virus in Adults Substantially Increases When Adding Sputum, Saliva, and Serology Testing to Nasopharyngeal Swab RT-PCR. Infect Dis Ther. 2023 Jun;12(6):1593-1603. doi: 10.1007/s40121-023-00805-1. Epub 2023 May 6. PMID: 37148463; PMCID: PMC10163290.

Adult Testing Meta-Analysis Conclusions

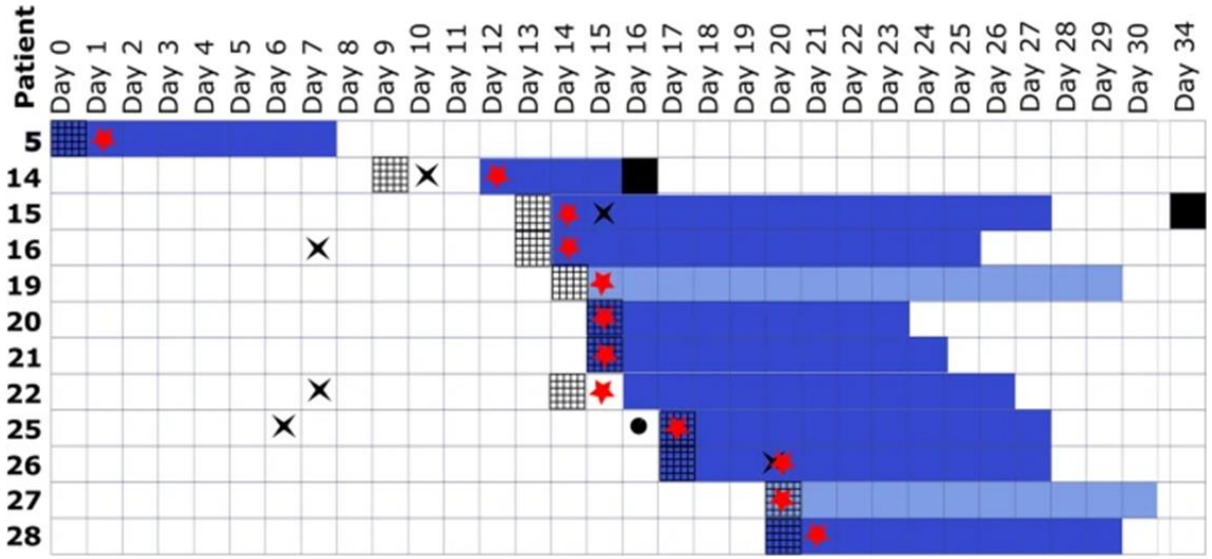
Adding specimen types such as paired serology and sputum to NP/nasal swab RT-PCR increased RSV detection by 50% to 66% on average, respectively

Suggest using ≥3 specimen types for robust analysis of prevalence in studies

Hospital Outbreaks

- French geriatric hospital outbreak
- 12 cases in 2 adjacent wards
- Mean age 89 – all had co-morbidities
- 2 passed away
- Genotyping confirmed these cases as an ‘outbreak’

Chronological illustration of clinical laboratory findings and implementation of droplets precautions for SPR-R and SPR-M RSV patients. n=19



Days relative to the start of the outbreak and indicated at the top. Solid bars indicate the droplet precaution period (SPR-R dark blue, SPR-M light blue). Black squares indicate patient death. Red stars indicate the laboratory confirmation of RSV infection. Grids indicate the onset of symptoms. Chest X ray (black cross) and physical therapy (black circle) are mentioned.

Hababou Y, Taleb A, Recoing A, Moreau F, Simon I, Muller de Schongor F, et al. Molecular investigation of a RSV outbreak in a geriatric hospital. BMC Geriatrics. 2021 Feb 12;21(1).

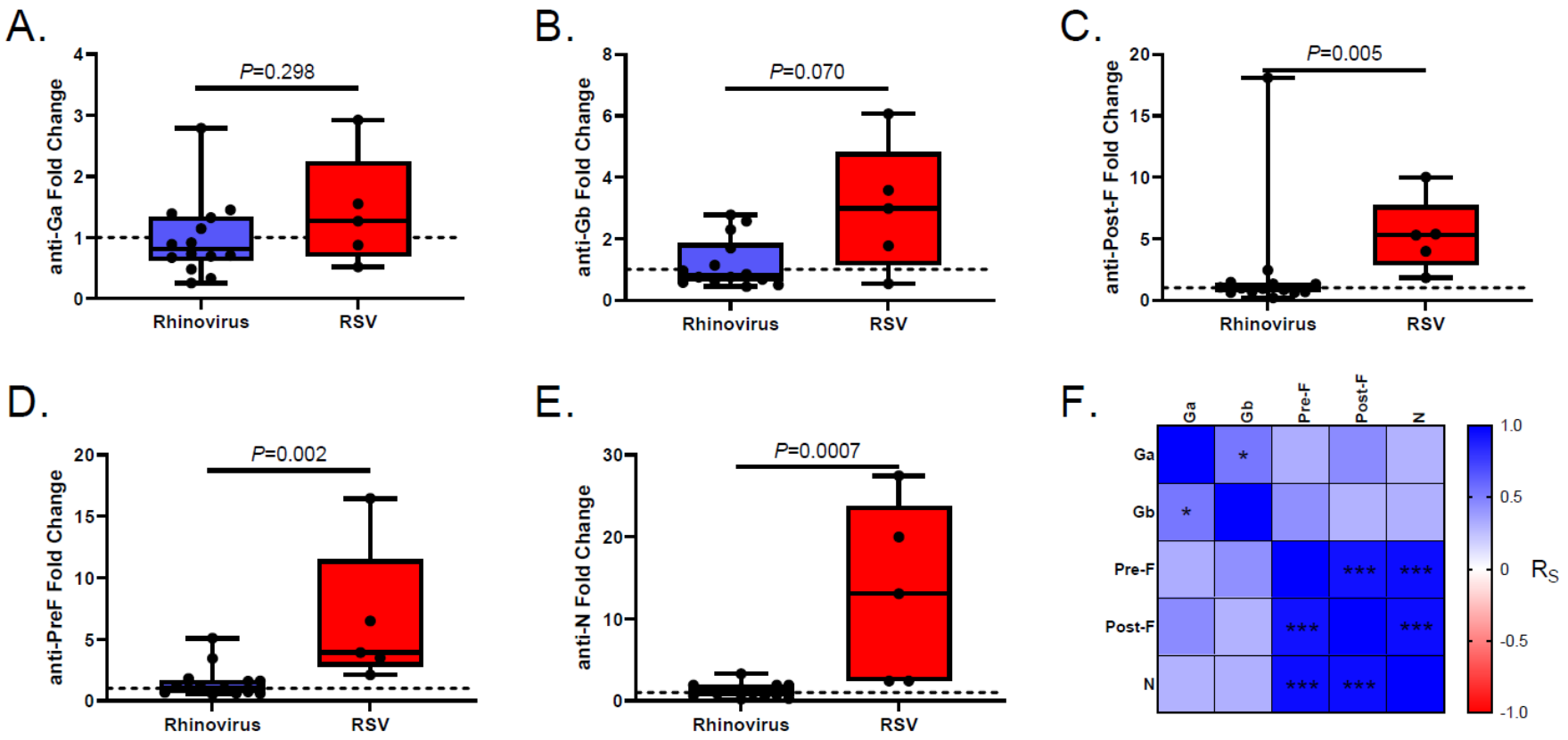
Whole Genome Sequencing

Possible vaccines could drive mutations

Large-scale genomic surveillance needed

Early detection of strains that escape intervention

The Future: Serology in Vaccinated Adults



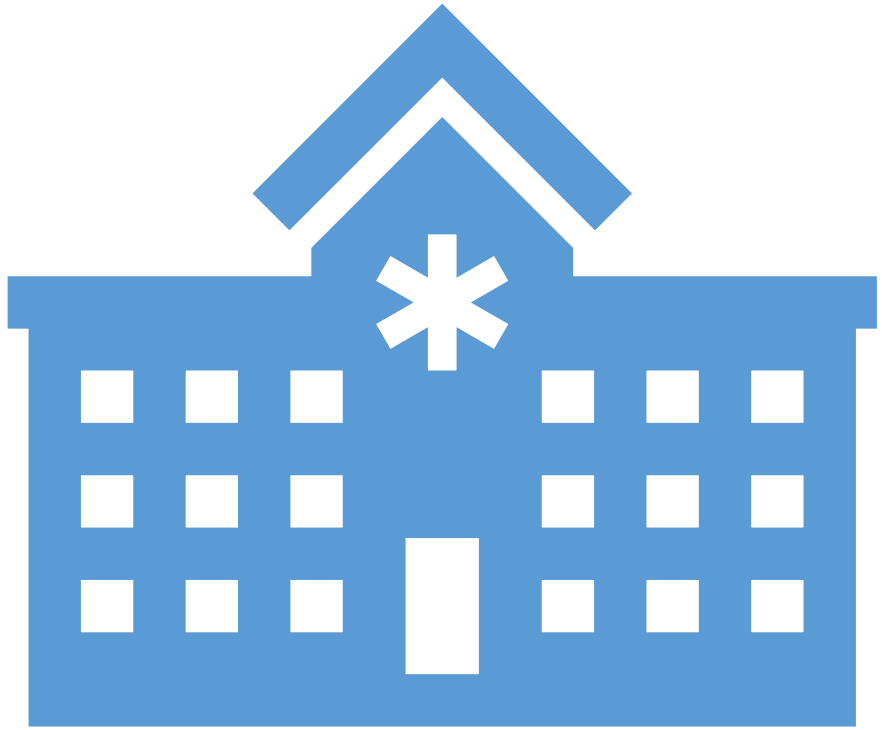
Wiseman, D et al Frequency of RSV-Related COPD Exacerbations Using Standard and Enhanced Diagnostic Methods: A Binational Prospective Cohort Study. Available at SSRN: https://ssrn.com/abstract=4487116 or http://dx.doi.org/10.2139/ssrn.4487116

Critical Issue:

Awareness of RSV in adults is lacking

Older Adult RSV Awareness

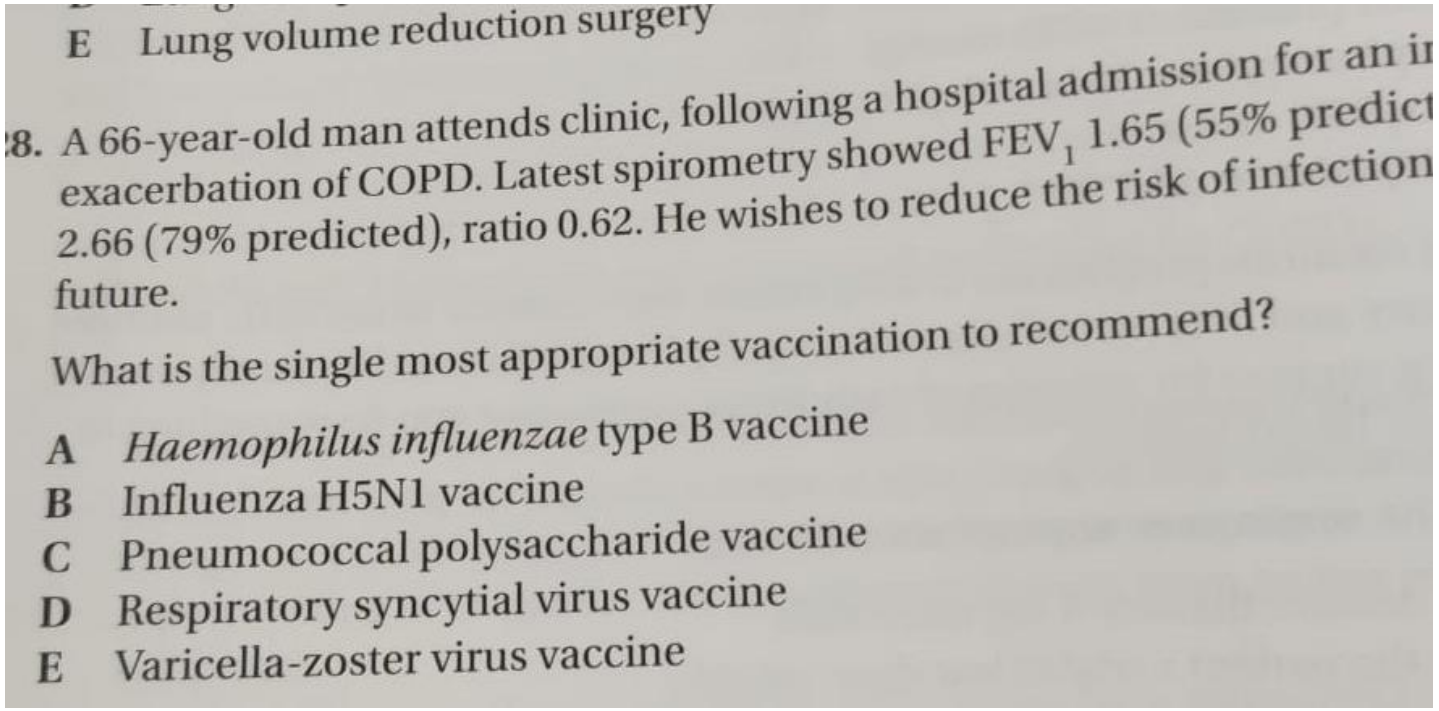
- In one study RSV was not considered on admission to A+E in most cases where it was eventually diagnosed. This included high risk patients. Only 36% of admitted patients eventually diagnosed with RSV were swabbed for viral PCR for RSV on admission.
- Among 827 of survey respondents, only 43.3% had ever heard of RSV



Elizabeth M. La, Su Bunniran, Diana Garbinsky, Maria Reynolds, Phil Schwab, Sara Poston & Lauriane Harrington (2024) Respiratory syncytial virus knowledge, attitudes, and perceptions among adults in the United States, Human Vaccines & Immunotherapeutics, 20:1, 2303796, DOI: 10.1080/21645515.2024.2303796

British Respiratory Specialists:

- **MRCP SCE in Respiratory Medicine: 300 SBAs, Second Edition**
- June 2023
- Publisher: Jaypee Brothers Medical Publishers Pvt Ltd
- ISBN: 1787791173

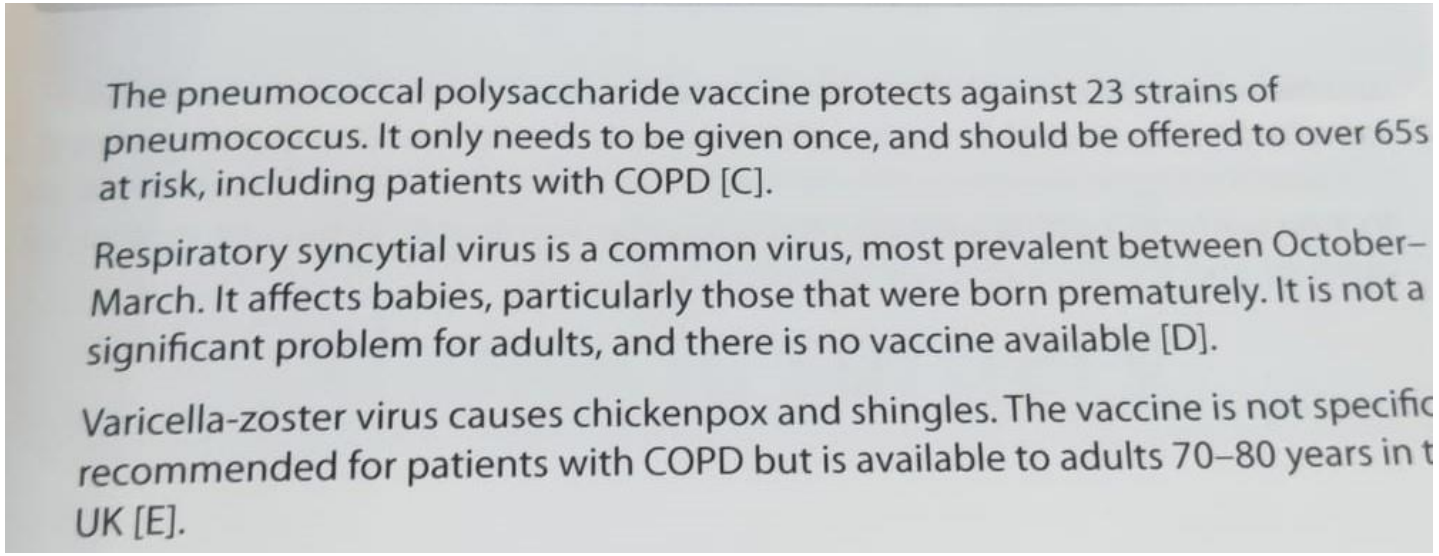


E Lung volume reduction surgery

8. A 66-year-old man attends clinic, following a hospital admission for an i exacerbation of COPD. Latest spirometry showed FEV_1 1.65 (55% predict 2.66 (79% predicted), ratio 0.62. He wishes to reduce the risk of infection future.

What is the single most appropriate vaccination to recommend?

A *Haemophilus influenzae* type B vaccine
B Influenza H5N1 vaccine
C Pneumococcal polysaccharide vaccine
D Respiratory syncytial virus vaccine
E Varicella-zoster virus vaccine



The pneumococcal polysaccharide vaccine protects against 23 strains of pneumococcus. It only needs to be given once, and should be offered to over 65s at risk, including patients with COPD [C].

Respiratory syncytial virus is a common virus, most prevalent between October–March. It affects babies, particularly those that were born prematurely. It is not a significant problem for adults, and there is no vaccine available [D].

Varicella-zoster virus causes chickenpox and shingles. The vaccine is not specific recommended for patients with COPD but is available to adults 70–80 years in t UK [E].

Take-Home Messages

- No assay has 100% accuracy
- Big differences in sensitivity between adult and infant populations
- Monoplex PCR is sensitive in diagnosing RSV in infants
- Adding extra methods will however increase diagnostics
- For adults, more methods are needed than just PCR
- Awareness of RSV in adults needs to improve
- Consider non-F-antigen based serology for a vaccinated cohort

Thank You!


Step 1.
Take a sample
(following instructions in section 16)
Step 2.
The swab is inserted into the
DnaCartridge - programmed for
Covid-19,RSV and Flu
Step 3.
The barcode on the
DnaCartridge is scanned by
the Capsule, and the Capsule
placed onto the NudgeBox
Step 4.
The NudgeBox runs the test
and sends the results to the
secure DnaNudge cloud
Step 5.
The cloud analyses the test data and
sends the results to the clinician via the
Operator App and/or standard hospital
or lab integration systems
Sample to answer :~1.5 hours