Testing Times – Diagnostics and POC Testing for RSV

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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.



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 <u>a</u>	-	+	-
995	-	-	-
Total no. of positives	43	87	104

^aDefined 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® R\$V 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	nª	% (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 adults¹



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)	58 (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	8 (17)	8 (14)	63 (10)
S _{pO2} <95%	5 (14)	10 (18)	39 (6)

	Health	ny, 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

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_{pQ} ; 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.

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.000000000000455

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	D	etection Ratio [95% CI]
Paired serology	/							
Zhang (2017)	< 18 years	Hospitalized or admitted	Community-acquired pneumonia	Nasopharyngeal swab	RT-PCR	1087	HH -1	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	ці.	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 estima	te (95% CI)						•	1.05 (.96–1.14)
Nasopharynge	al 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	H	1.00 (.67-1.49)
Pooled estima	te (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	H=	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	rii	1.10 (.74-1.63)
Pooled estima	te (95% CI)						•	1.08 (.94–1.25)
						[
						0.25 0	.5 1 2	
						Detection	n Ratio [95% CI]	

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, <u>https://doi.org/10.1093/infdis/jiad185</u>

POC vs RT-PCR in infants

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

A							
Study ID	Index platform	Sample type	TP	FN + TP		Sensitivity	95% CI
Studies in children 0–1 years Miemyk (2011)	Binax NOW RSV	Nasopharyngeal aspirate	46	58		0.79	[0.67; 0.89]
Studies In children 0–5 years Freeman (2021) Knanom (2011) Memyk (2011) Moessker (2016) Papenburg (2013) Schauer (2016) Yaz-de-Lina (2008) Vaz-de-Lina (2008) Viguria (2017) Pooled estimates (65% CI) Heterogeneity: F= 98%, p < 0.01	Quidel QuickView BinaxNOW RSV BinaxNOW RSV BinaxNOW RSV Directigen RSV, Abott Test Pack, Pathfinder RSV ELISA Kit B0 Vertior RSV PCC assay B0 Directigen EZ RSV kit BinaxNOW RSV	Nasal swab, Cropharyngeal swab Nasopharyngeal swab Nasopharyngeal saprate Nasal wash Nasopharyngeal aspirate Nasopharyngeal saprate Nasopharyngeal saprate Nasopharyngeal swab	208 21 11 162 370 411 146 105 94	810 51 204 463 621 179 132 198 2679	* *****	0.26 0.41 0.52 0.79 0.80 0.66 0.82 0.80 0.47 0.63	[0.23; 0.29] [0.28; 0.56] [0.30; 0.74] [0.73; 0.85] [0.62; 0.70] [0.52; 0.70] [0.75; 0.87] [0.72; 0.86] [0.40; 0.55] [0.49; 0.76]
Studies in children 0–18 years Aslanzach (2008) Beil (2014) Beil (2014) Beil (2014) Beil (2014) Brottors (2014) Grödnahi (2005) Ivaska (2013) Jonchheert (2015) Vertil (2015) Kurtowa (2004) Kurtowa (2004) Kurtowa (2004) Schützle (2005) Shrato (2014) Schützle (2005) Walsh (2015) Heatorgoanels// /² = 65%, ρ < 0.01	BD Directigen EZ RSV kit BD Verior system BD Verior system mafPCC assay TestPack RSV mafPCC assay TestPack RSV mafPCC assay TestPack RSV SAS RSV Directigen EZ RSV SAS RSV Directigen EZ RSV SOFIATM-RSV BD TestCigen RSV	Nasopharyngeal aspirate, wash Nasopharyngeal aspirate, Nasopharyngeal swab Nasopharyngeal aspirate, Nasopharyngeal swab Nasopharyngeal aspirate, Nasopharyngeal swab Nasopharyngeal aspirate Nasopharyngeal aspirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal sapirate	190 296 145 151 80 48 31 121 121 121 121 49 66 55 65 15 63 25 114 204	219 392 199 102 62 31 153 153 153 81 79 79 88 108 29 145 257 2385	┈┸┱╪┿╄ [┯] ┱╪┿╄ [┿] ╋┿┿╸ ╻╌┙╾╕	0.87 0.76 0.78 0.78 0.77 1.00 0.79 0.60 0.84 1.00 0.74 1.00 0.74 0.79 0.79 0.79 0.79 0.79 0.79 0.79 0.79	(0.82: 0.91) (0.71: 0.80) (0.66: 0.79) (0.72: 0.84) (0.63: 0.86) (0.63: 0.86) (0.63: 0.86) (0.72: 0.85) (0.72: 0.85) (0.74: 0.85) (0.74: 0.91) (0.74: 0.84) (0.74: 0.84) (0.74: 0.84) (0.74: 0.82)
Pooled estimates (95% CI) Heterogeneity: l^2 = 96%, ρ < 0.01 Test for subgroup differences: χ_2^2	= 5.35, df = 2 (p = 0.07)			5122 0	0.2 0.4 0.6 0.8 1	0.75	[0.69; 0.80]
Watapharyngeal anab, Gropheryngeol anab, BA	expirate, Enclotracheal aspirate			Sen	sitivity (RADT vs RT-PCR)		
В							
B Study ID	Index platform	Sample type	TN	FP + TN	,	Specificity	95% CI
B Study ID Studies in children 0-1 years Miemyk (2011)	Index platform Binax NOW RSV	Sample type Nasopharyngeal aspirate	TN 150	FP + TN 155		Specificity 0.97	95% CI [0.93; 0.99]
B Studies in children 0-1 years Memryk (2011) Studies in children 0-5 years Freeman (2021) Khanom (2011) Memryk (20110) Papenburg (2013) Schwarz (2015) Schwarz (2015) Vaz-de-Lima (2008) Viguria (2017) Pooled estimates (95% C1) Hoterogeneity: F = 95%, p. C 01	Index platform Binax NOW RSV Undel OurkK/ww BinaxNOW RSV BinaxNOW RSV BinaxNOW RSV Direction RSV, Abbott. Test Pack, Pathfinder RSV ELISA Kit. B) Verior RSV PCC assay B) Direction EZ RSV kit.	Sample type Nasopharyngeal aspirate Nasopharyngeal swab Nasopharyngeal swab Nasopharyngeal aspirate Nasopharyngeal aspirate Nasopharyngeal sapirate Nasopharyngeal sapirate Nasopharyngeal swab	TN 150 458 108 76 305 249 880 341 166 137	FP + TN 155 461 108 77 310 257 979 344 174 223 2933	# 	0.97 0.99 1.00 0.99 0.96 0.97 0.90 0.90 0.95 0.61 0.98	95% C1 (0.98; 1.00) (0.97; 1.00) (0.97; 1.00) (0.94; 0.99) (0.94; 0.99) (0.94; 0.99) (0.97; 1.00) (0.97; 1.00) (0.97; 0.98) (0.95; 0.68) [0.93; 0.99]
B Study ID Studies in children 9–1 years Mierryk (2011) Studies in children 9–5 years Freeman (2021) Mierryk (2011) Mierryk (2011) Mierryk (2011) Mierryk (2011) Mierryk (2011) Schwartz (2015) Schwartz (2015) Vaz-de-Lima (2028) Vaz-de-Lima (2028) Vaz-de-Lima (2028) Mierryk (2017) Pooled estimates (95% C1) Heitergeneity: F = 95%, e / C1) Bei (2014) Bei (2014) Bei (2014) Bei (2014) Bei (2015) Vasta (2015) Vasta (2015) Vasta (2015) Vasta (2015) Vasta (2015) Vasta (2015) Vasta (2015) Vasta (2015) Vasta (2014) Schütze (2005) Nata (2007) Tuttle (2015) Nata (2014) Pooled estimates (95% C1) Heorogeneity: <i>F</i> = 94%, e/C011	Index platform Binax NOW RSV Quidel QuickView BinaxNOW RSV BinaxNOW RSV BinaxNOW RSV BinaxNOW RSV Directigan FSV, Abbott Test Pack, Pathfinder RSV ELISA Kit DS Vrinior RSV PoCC assay BD Directigan EZ RSV kit BD Metric system CAT BinaxNOW RSV BD Directigan EZ RSV kit BD Metric system RSV KSF BD Vertior System RSV KSF BD Devetion RSV RSV SOFIATM-RSV FIA Directigen RSV RSV SOFIATM-RSV FIA Directigen RSV	Sample type Nasopharyngeal aspirate Nasopharyngeal aspirate Nasopharyngeal sayntae Nasopharyngeal sayntae	TN 150 458 76 305 249 880 1337 248 667 1216 133 241 166 1337 248 451 216 133 234 212 232 234 313 234 264 160 8235	FP + TN 155 461 108 77 207 979 344 174 223 2333 296 676 475 419 127 95 285 285 285 295 235 35 292 17 541 310 27 55 431 419 55 285 285 295 417 419 55 285 285 295 285 285 285 285 285 285 285 28	* * ****	Specificity 0.97 100 0.98 0.97 0.98 0.95 0.95 0.95 0.95 0.95 0.95 0.95 0.95	95% C1 [0.93; 0.99] (0.98; 1.00] (0.97; 1.00] (0.97; 1.00] (0.97; 1.00] (0.93; 0.99] (0.97; 1.00] (0.97; 1.00] (0.97; 1.03] (0.97; 0.86] (0.93; 0.99] (0.93; 0.99] (0.96; 1.00] (0.96; 0.99] (0.96; 0.99] (0.96; 0.99] (0.96; 0.99] (0.97; 0.99] (0.96; 0.99] (0.96; 0.99] (0.97; 0.99] (0.96; 0.99] (0.96; 0.99] (0.97; 0.97] (0.97; 0

Multiplex PCR is less sensitive than monoplex in infants

Virus	Concentration [copies/ml]	RVP	RVP			Respir	inder-1	9		Respire	inder- S	MART-2	2	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.

		Resplex	II Panel		ResPlex II Plus Panel PRE			ResPlex II Plus Panel PRE			. 2	D	
Viene		No. of sp	ecimens		Sensitivity (%)		No. of sp	ecimens		Sensitivity	χ¯ value	P value	
virus	M+R+	M+R-	M-R+	M-R-	(70)	M+R+	M+R-	M-R+	M-R-	(70)	value	varue	
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	

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

* 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). <u>https://doi.org/10.1007/s12250-013-3312-y</u>

• 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, <u>https://doi.org/10.1093/cid/ciaa648</u>



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 rollout 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 (≤10³ versus ≤10⁶ PFU/ml)



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%.

Α								
Study ID	Index platform	Summary age		TP F	N + TP		Sensitivity	95% CI
Age group = Mixed							1	
Bierbaum (2014)	Fast-track diag RPA	6 (1-59)	Median (IQR)	65	68			[0.88; 0.99]
Eigner (2019)	Cobas omni UCS	NR	NR	69	70		0.99	[0.92; 1.00]
Jokela (2012)	xTAG RVP Fast	NR	NR	55	59		0.93	[0.84: 0.98]
Sawatwong (2013)	TagMan Array Card	NR	NR	9	10		0.90	[0.55; 1.00]
Yan (2017)	multiplex LiquiChip assay (rMLA)	NR	NR	37	39		0.95	[0.83; 0.99]
Yan (2017)	TaKaRa PrimeScript RT-PCR	NR	NR	35	39	50-	0.90	[0.76; 0.97]
Random effects model					285		• 0.95	[0.91; 0.97]
Heterogeneity: $l^2 = 0\%$, $p = 1$	0.54							8
Age group = Adults (18)	years and over)							
Svensson (2014)	Simplexa Flu A/B & RSV	NR	NR	46	57		- 0.81	[0.68; 0.90]
Random effects model					342		0 0 93	10 88- 0 961
Haterogeneity: $l^2 = 54\%$, n =	0.04					1 1 1 1	0.55	[0.00, 0.30]
Test for subgroup difference	$s_{1}^{2} = 11.64 \text{ df} = 1 (n < 0.01)$				0	02 04 06 08	1	
reactor subgroup universes	α. χ ₁ - 11.04, αι - 1 (β - 0.01)			Se	nsitivity (Mu	ultiplex RT-PCR vs Sing	leplex RT-PCR)	
				00	ioning (inc	unproxiter i officio offig		
В								
Study ID	Index platform	Summary age		TN F	P + TN		Specificity	95% CI
Age group = Mixed							1	
Bierbaum (2014)	Fast-track diag RPA	6 (1-59)	Median (IQR)	296	301			[0.96; 0.99]
Eigner (2019)	Cobas omni UCS	NR	NR	300	301		1.00	[0.98; 1.00]
Jokela (2012)	xTAG RVP Fast	NR	NR	233	238		0.98	[0.95; 0.99]
Sawatwong (2013)	TaqMan Array Card	NR	NR	80	80		1.00	[0.95; 1.00]
Yan (2017)	multiplex LiquiChip assay (rMLA)	NR	NR	34	35		0.97	[0.85; 1.00]
Yan (2017)	TaKaRa PrimeScript RT-PCR	NR	NR	33	35		0.94	[0.81; 0.99]
Random effects model					990		 0.99 	[0.97; 0.99]
Heterogeneity: $l^2 = 14\%$, $p =$	0.32							
Age group = Adults (18)	years and over)							
Svensson (2014)	Simplexa Flu A/B & RSV	NR	NR	153	153			[0.98; 1.00]
Random effects model					1143		< 0.99	[0.98; 1.00]
Heterogeneity: $I^2 = 0\%$, $p = 0$	0.44					1 1 1 1 1		
Test for subgroup differences	s: $\chi_1^2 = 0.00$, df = 1 (p = 1.00)				0.7	0.75 0.8 0.85 0.9 0.9	95 1	
				Sp	ecificity (Mu	ultiplex RT-PCR vs Sing	leplex RT-PCR)	

On wuchekwa 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.

Α	Study ID Inc	lex platform	Summary age		TP	FN + TP	•	Sensitivity	95% CI
	Age group = Mixed	M Disgradies	ND	ND	50	57	. 1	- 0.01	10 81-0 071
	Jokela (2012) Lig	Int Diagnostics	NR 40	NR	22	255	1	0.91	[0.81, 0.97]
	Kuypers (2006) NA		10	Median	231	200		= 0.93	[0.69, 0.96]
	Landry (2014) Cyt	ospin-ennanced DFA	NR	NR	34	40		0.74	[0.59, 0.86]
	Heterogeneity: $l^2 = 86\%$, $p < 0.01$					358		U.88	[0.78; 0.94]
	Age group = Adults (18 years and over)								
	Simpson (2003) Vie	das RSV	16-74	Range	10	18	· ·····	0.56	[0.31; 0.78]
	Random effects model					376		- 0.83	[0.66; 0.93]
	Heterogeneity: $I^2 = 89\%$, $p < 0$ Test for subgroup differences:	$x^2 = 8.40 \text{ df} = 1 (n < 0.01)$					0 02 04 06 08	1	
	rearior subgroup differences.	χ ₁ = 0.40, αι = 1 (μ = 0.01)					Sensitivity (DFA vs RT-PC	R)	
С	Study ID	Index platform	Summary age		TP I	FN + TP		Sensitivity	95% Cl
	Age group = Miyed						1		
	Runing (2017)	MariPOC	32 (13.9.55)	Modian (IOP)	6	12	198	0.50	10 21 0 701
	Comer (2016)	Sofia DSV	ND	Median (iGR)	128	120		0.00	[0.21, 0.75]
	Gontez (2010)	Direction DC1/	NR	NE	120	70	100	0.92	[0.00, 0.90]
	Goodinci (2007)	Direcigen Kov	10 1 (21 5)	Maga (CD)	4/	19	100	0.59	[0.46, 0.70]
	Jung (2016)	BD ventor system	18.1 (31.5)	Mean (SD)	101	160	1000	0.63	[0.55; 0.71]
	Jung (2016)	BINAXNOW RSV	18.1 (31.5)	Mean (SD)	99	160	100	0.62	[0.54; 0.69]
	Jung (2016)	Humasis	18.1 (31.5)	Mean (SD)	108	160	Tan	0.68	[0.60; 0.75]
	Jung (2016)	SD RSV BIO LINE	18.1 (31.5)	Mean (SD)	101	160		0.63	[0.55; 0.71]
	Leonardi (2015)	Directigen RSV	NR	NR	28	40		0.70	[0.53; 0.83]
	Leonardi (2015)	Quickvue RSV	NR	NR	23	40		0.58	[0.41; 0.73]
	Leonardi (2015)	Sofia RSV	NR	NR	34	40		- 0.85	[0.70; 0.94]
	Leonardi (2015)	Veritor system	NR	NR	29	40		0.72	[0.56; 0.85]
	Sanbonmatsu-Gamez (201	5) mariPOC	NR	NR	38	45		- 0.84	[0.71; 0.94]
	Random effects model	0.01				1075	0	0.71	[0.63; 0.78]
	reserve $(1 - 1 + 2 + 2 + 2 + 2 + 2 + 2 + 2 + 2 + 2 +$								
	Age group = Adults (18 y	ears and over)							
	Franck (2020)	BinaxNOW RSV	52 (35-69)	Median (IQR)	8	57		0.14	[0.06; 0.26]
	Franck (2020)	RSV ImmuView	52 (35-69)	Median (IQR)	13	57		0.23	(0.13: 0.36)
	Random effects model		02 (00 00)	mountificary		114	0	0.18	10.12:0.271
	Heterogeneity: $I^2 = 30\%$, $p =$	0.23							ferred everly
	Random effects model					1189		0.64	[0.50: 0.75]
	Heterogeneity: $l^2 = 90\% \rho < 0.01$					Г	1 1 1 1		
	Test for subgroup differences: $y^2 = 59.85$ df = 1 ($a < 0.01$)					0	02 04 06 08	1	
	tear of surgroup differences	. Al - 00.00, 01 - 1 (b < 0.01)				U	Sensitivity (RADT vs RT-PC	R)	

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

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 Pros pective 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



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

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 \geq 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 comorbidities
- 2 passed away
- Genotyping confirmed these cases as an 'outbreak'



Chronological illustration of clinical laboratory findings and implementation of droplets precautions for

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.

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: <u>https://ssrn.com/abstract=4487116</u> or <u>http://dx.doi.org/10.2139/ssrn.4487116</u>

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, PhilSchwab, Sara Poston & La uriane Harrington (2024) Respiratory syncytial virus knowledge, attitudes, and perceptions among a dults 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
 E Lung volume reduction surgery
 8. A 66-year-old man attends clinic, following a hospital admission for an in exacerbation of COPD. Latest spirometry showed FEV₁ 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 to 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

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



Thank You!

