

Underascertainment of Respiratory Syncytial Virus Infection in Adults Due to Diagnostic Testing Limitations: A Systematic Literature Review and Meta-analysis

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Background. Most observational population-based studies identify respiratory syncytial virus (RSV) by nasal/nasopharyngeal swab reverse transcriptase real-time PCR (RT-PCR) only. We conducted a systematic review and meta-analyses to quantify specimen and diagnostic testing-based underascertainment of adult RSV infection.

Methods. EMBASE, PubMed, and Web of Science were searched (January 2000–December 2021) for studies including adults using/comparing >1 RSV testing approach. We quantified test performance and RSV detection increase associated with using multiple specimen types.

Results. Among 8066 references identified, 154 met inclusion. Compared to RT-PCR, other methods were less sensitive: rapid antigen detection test (RADT; pooled sensitivity, 64%), direct fluorescent antibody (DFA; 83%), and viral culture (86%). Compared to singleplex PCR, multiplex PCR's sensitivity was lower (93%). Compared to nasal/nasopharyngeal swab RT-PCR alone, adding another specimen type increased detection: sputum RT-PCR, 52%; 4-fold rise in paired serology, 44%; and oropharyngeal swab RT-PCR, 28%. Sensitivity was lower in estimates limited to only adults (for RADT, DFA, and viral culture), and detection rate increases were largely comparable.

Conclusions. RT-PCR, particularly singleplex testing, is the most sensitive RSV diagnostic test in adults. Adding additional specimen types to nasopharyngeal swab RT-PCR testing increased RSV detection. Synergistic effects of using ≥ 3 specimen types should be assessed, as this approach may improve the accuracy of adult RSV burden estimates.

Keywords. respiratory syncytial virus infections; adults; diagnosis; epidemiology; sensitivity and specificity.

Key summary. Respiratory syncytial virus (RSV) is an important cause of illness and death among older adults. Most studies of how frequent RSV infection is among older adults use only nasal swab testing to identify RSV infection. These nasal swabs are checked for genetic material from the virus, known as polymerase chain reaction (PCR) testing. We examined published studies from January 2000 to December 2021 to estimate how many RSV infections would be missed by using only this approach to RSV testing. We found 154 studies had information to answer our question. Compared to PCR testing of nasal swab alone, adding sputum specimen PCR testing (ie, testing cough mucus or phlegm for RSV genetic material) increased RSV infections found by 52%. Adding blood testing increased RSV infections found by 44%. Adding mouth/throat swab PCR testing, increased RSV infections by 28%. In summary, adding additional specimen types to nasal swab PCR testing increased RSV detection. Impact of using 3 or more specimen types at the same time should be assessed, as this approach may further improve accuracy.

Respiratory syncytial virus (RSV) causes acute respiratory infection in persons of all ages. The Global Burden of Disease Study 2016 reported 25 million episodes of RSV infections

and 77 000 associated deaths worldwide [1]. While mild in most healthy adults, RSV can result in severe illness and poor outcomes in persons who are frail, elderly, or have underlying health conditions [2].

Due to RSV's nonspecific clinical manifestations, laboratory testing of respiratory secretions is required to confirm infection [3]. In adults, low viral titers and shorter duration of viral shedding (compared to young children) limit RSV detection using current diagnostic modalities [2].

Historically, the gold standard diagnostic modality was viral culture but this has been largely replaced by more sensitive and less time-consuming methods [4]. Molecular techniques, such as nucleic acid amplification tests, which can detect very low viral titers, have improved test sensitivity in adults and produce

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results more quickly. Reverse transcriptase real-time polymerase chain reaction (RT-PCR) has become the reference diagnostic method for RSV detection due to its high diagnostic accuracy [3, 5].

A rise in serology titers (≥ 4 -fold rise between acute/convalescence or pre-/postseason specimens) can identify recent RSV infection although some adults do not seroconvert [6, 7]. This method is largely limited to epidemiological studies because a retrospective diagnosis is less useful for clinical care [5]. Alternative diagnostic methods such as antigen detection assays (eg, direct fluorescent antibody [DFA] tests and rapid antigen detection tests [RADTs]) offer fast results and are easy to perform but have low sensitivity in adults [4, 5].

While RT-PCR increases RSV detection sensitivity compared to other testing modalities, the specimen used for diagnostic test can impact sensitivity of RSV detection. Specimen collection using nasopharyngeal swabs (NPS), although better tolerated than nasal wash or nasopharyngeal aspirate (NPA) in adults, yields lower viral titers and underestimates RSV infections [3, 5]. Studies combining sputum and paired serology with NP/nasal swab have further documented the underascertainment associated with single specimens, particularly NPS [7].

As a result of the diagnostic tests employed and the choice of specimen used, many RSV infections are likely missed. This in turn has implications for designing public health policies as these are heavily dependent on accurate determination of disease burden [3, 5]. Furthermore, underascertainment of RSV infections reduces statistical power and may lead to misclassification within vaccine efficacy trials, thus impeding vaccine development efforts [8].

We conducted a systematic literature review and meta-analysis to collate comparative literature on RSV diagnostic testing in adults and quantify the underascertainment associated with RSV diagnostic testing and sampling approaches. This quantification could allow adjustment of clinical burden estimates for diagnostic testing-related underascertainment and thus better estimate the true burden of disease.

METHODS

Protocol and Registration

This systematic review protocol adhered to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) for Diagnostic Test Accuracy (DTA) Studies [9] and is registered on the PROSPERO database (registration No. CRD42022313209).

Study Eligibility Criteria

Eligibility criteria were developed using the Population-Intervention-Comparator-Outcomes-Time Frame-Setting (PICOTS) framework (Supplementary Table 1). Briefly, primary studies were

eligible if they reported on the diagnostic test performance or compared RSV detection rates using different specimens. We excluded secondary research (eg, reviews, meta-analyses), studies in children, and in vitro studies.

Information Sources and Search Strategy

Embase, MEDLINE (via PubMed), Web of Science, and nonindexed sources and RSV research networks (Supplementary Table 2) were searched for publications during 1 January 2000 to 27 December 2021. Additional relevant articles were sought by manual search of systematic reviews' bibliographies. Supplementary Table 3 presents detailed search strategies.

Study Selection

Retrieved articles were screened based on a priori eligibility criteria (Supplementary Table 1). First, titles and abstracts were screened to identify potentially relevant articles. Subsequently full-text screening was performed in duplicate by independent reviewers (L.M.M., B.M.) with a third reviewer resolving disagreements (C.O., S.M.).

Data Extraction

Predefined data items were extracted from each included article into a piloted structured extraction form on DistillerSR [10]. One reviewer extracted the data while a second reviewer cross-checked the collected data. Discrepancies were resolved by consensus or a third reviewer acted as an arbiter. Data items extracted for this review are summarized in the Supplementary Table 4.

For this review, we abstracted data on paired specimens (hence ≥ 2 specimen types from the same patient using the same diagnostic test) or paired testing (1 specimen type using ≥ 2 diagnostic methods). Overall RSV detection was defined as the number of specimens that tested positive for RSV by any testing approach. Consistent with PRISMA-DTA guidance, the index test was defined as the test under evaluation while the reference test was the comparator test [9].

Risk of Bias and Applicability

Study quality assessment was performed by one reviewer and verified by a second reviewer using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria [11]. Disagreement between reviewers' assessments was resolved by consensus and, if required, a senior review team member resolved the conflict. To allow assessment of studies comparing RSV detection by different specimens, we included a domain to capture potential sources of bias due to specimen collection and flow. In Supplementary Table 5, we outline each domain component, highlighting how we made judgements concerning risk of bias.

Diagnostic Accuracy Measures and Synthesis of Results

Characteristics of studies and populations were analyzed and reported descriptively. We reported the overall detection rate (DR) and test-specific DR for each study. Subsequently, the DR ratio (DRR) was estimated as (positive by any method)/(positive by reference test). RT-PCR and NPS and nasal swabs were considered reference test and sampling methods, respectively. We chose NPS and nasal swab combined as the gold standard because they were the most commonly used tests for adult RSV detection in identified studies and because evidence from influenza and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) suggests that they may perform similarly [12, 13].

Data from 2-by-2 tables were used to measure diagnostic tests' sensitivity and specificity, calculate 95% confidence intervals (CIs), and were then presented graphically in a forest plot. For this review, sensitivity is defined as the proportion of RSV-positive specimens correctly identified, and specificity as the proportion of RSV-negative specimens correctly identified by a test against a reference. Diagnostic test performance summary is presented separately for RADTs, DFA, viral culture, and serology using any RT-PCR type, including singleplex, multiple, in-house, and commercial platforms, as a common reference and stratified by age groups. Age groups were adults only and combined results for adults and children where no stratification was reported. Meta-analyses of each diagnostic test's performance were performed where applicable using random-effects models. Additionally, we evaluated the performance of multiplex RT-PCR (targeting multiple respiratory pathogens) against singleplex RT-PCR (only RSV), and the concordance between multiplex RT-PCR platforms. All analyses were performed in R version 4.2.0, using the meta4diag and metafor package.

Sensitivity Analyses

Given the potentially higher sensitivity of diagnostic tests among children, a sensitivity analysis was conducted to explore whether results were affected by excluding studies involving both adults and children (mixed population) or not reporting the percentage of adults (unspecified population).

RESULTS

Study Selection

Our database search returned 9219 references and 690 references were identified from other sources (Supplementary Table 2), producing 8066 unique references. After screening, we included 154 articles in the review (Figure 1). Two additional articles were included based on expert recommendation (E.B.) [14, 15].

Study Characteristics

Supplementary Table 6 provides the complete list and detailed description of included studies. Most were conducted in North

or South America (37%) or Europe (32%), and 25 studies included only adults (Table 1). Most studies had a cross-sectional design (137/154), while 8 were prospective surveillance studies, and 9 used other designs.

Overall, 77 studies recruited patients with lower respiratory tract infection or exacerbation of an underlying cardiac or pulmonary disease, while 55 studies included patients with upper respiratory tract infection. When reported (59 studies), study populations were most frequently hospitalized patients (66%).

The most common upper respiratory samples were NPS (102 studies), NPA (48 studies), and oropharyngeal swab (OPS, 36 studies). The most frequently used diagnostic approach was RT-PCR, with 68.5% (113 studies) using multiplex platforms and 15.8% (26 studies) using singleplex platforms. DFA testing and RADTs were reported in 20 studies and 18 studies, respectively, while 6 studies evaluated paired serological testing.

Risk of Bias and Applicability

Overall, 99 of 154 (64.3%) included studies were graded as having a high risk of bias in ≥ 1 domain (46% for primary end point, single-sample RT-PCR vs combined testing approach). Quality assessment is presented in Figure 2 and Supplementary Table 7.

In the patient selection domain, 74 studies (48.7%) had an unclear risk of bias due to lack of clear reporting of patient selection process and study setting (31% for primary end point) and 44 (29%) were graded as high risk of bias. In the index test domain, 51 studies (33.1%) had a high risk of bias due to either unblinded operators or lack of predefined threshold or internal control. While in the reference standard domain, 33 studies (21.4%) were assessed as high risk and 48 studies (31.1%) as unclear risk of bias. In the flow and timing domain, 51 studies (33%) were judged to be at high risk of bias. All 13 studies where our QUADAS-2 extension was used had low risk of bias due to sample flow.

For applicability assessment, 20 studies (13%) had patient selection domain risks, mostly studies without patient information or with inclusion of children without age-stratified results. In other domains, the number of studies with concerns were low.

Results of Individual Studies

The number of RSV infections detected overall, and by specific testing methods in each study, is presented in Supplementary Table 8.

RSV Detection by Single Sample RT-PCR Versus Combined Testing Approach

We identified 13 studies comparing RSV detection using RT-PCR based on NPS or nasal swab alone versus a combined testing approach [14–26]. The additional samples and testing approach included: paired serology testing (6 studies),

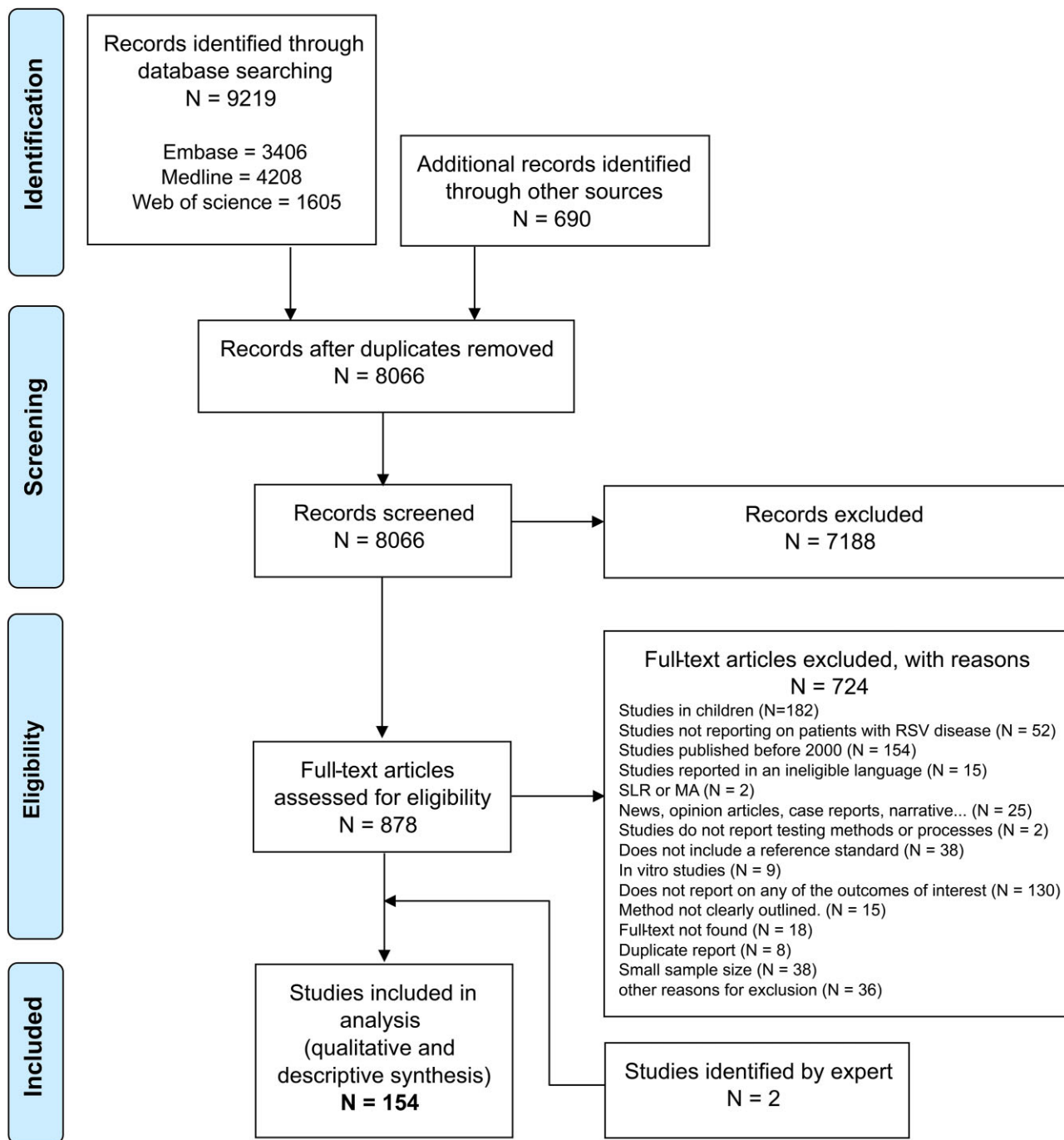


Figure 1. PRISMA flow chart showing the study selection process. Abbreviations: MA, meta-analyses; SLR, Systematic literature reviews.

RT-PCR on OPS (3 studies), sputum (3 studies), saliva (2 studies), and NPA (1 study) (Table 2).

The addition of sputum RT-PCR to NP/nasal swab RT-PCR increased RSV detection by 52% (DRR, 1.52; 95% CI, 1.15–2.01), while adding RT-PCR of OPS increased detection by 28% (DRR, 1.28; 95% CI, 1.11–1.49) (Figure 3). Compared to RT-PCR on a NP/nasal swab sample only, the addition of

paired serology testing increased detection by 42% (DRR, 1.42; 95% CI, 1.19–1.70).

Diagnostic Accuracy of Tests

We conducted meta-analyses of diagnostic test performance on index tests against a common reference of RT-PCR (Supplementary Table 9).

Table 1. Summary of Key Characteristics of Included Studies

Characteristic	No. (%) (n = 154)
World Health Organization region	
Americas	59 (37)
Europe	53 (32)
Western Pacific	38 (24)
Africa	3 (1.9)
South-East Asia	3 (1.9)
Study design	
Cross-sectional	137 (89)
Surveillance	8 (5.2)
Prospective cohort	4 (2.6)
Case-control	3 (1.9)
Retrospective cohort	1 (0.6)
Randomized controlled trial	1 (0.6)
Age group	
Adults and children	80 (52)
Adults, 18 y and older	25 (16)
Not reported, eg, based on stored samples	49 (32)
Clinical setting	
Inpatient only, hospitalized and ICU	29 (19)
Emergency department only	7 (4.5)
Outpatient only, clinics and primary care centers	8 (5.2)
Inpatient and emergency department, mixed	3 (1.9)
Outpatient, inpatient, and emergency department, mixed	8 (5.2)
Others, community-based and clinical trial sites	4 (1.9)
Not reported	95 (62)
Clinical presentation of the population studied	
LRTI only ^a	30 (19.5)
URTI only	13 (8.4)
URTI or LRTI	42 (27.3)
LRTI exacerbating preexisting conditions	5 (3.2)
Other ^b	3 (1.9)
Not reported	61 (39.6)
Specimen type^c	
Nasopharyngeal swab	102 (47.6)
Nasopharyngeal aspirate	49 (31.8)
Bronchoalveolar lavage	40 (18.7)
Oropharyngeal swab	36 (16.8)
Others ^d	27 (12.6)
Sputum	23 (10.7)
Nasal swab	19 (12.3)
Nasal wash/aspirate	12 (5.6)
Paired serology sample	6 (2.8)
Not specified	3 (1.4)
Saliva	2 (0.6)
Diagnostic test	
Multiplex RT-PCR	113 (68.5)
Singleplex RT-PCR	26 (15.8)
Viral culture	36 (21.8)

Table 1. Continued

Characteristic	No. (%) (n = 154)
Direct fluorescent antibody test	20 (12.1)
Rapid antigen detection tests	18 (10.9)
Others ^e	10 (6.5)
Conventional PCR	6 (3.6)
Serology	6 (3.6)

Abbreviations: ICU, intensive care unit; LRTI, lower respiratory tract infection; RT-PCR, reverse transcription-polymerase chain reaction; URTI, upper respiratory tract infection.

^aIncludes pneumonia, acute bronchitis, bronchiolitis, and influenza-like illnesses.

^bPatients with respiratory illness or suspected respiratory illness with unclear category.

^cEighty studies used a combination of respiratory samples for testing.

^dUnspecified upper respiratory swab, stored nucleic acid extract, unspecified respiratory secretions, pleural fluid, mouth wash, endotracheal tube specimen, and bronchial brushings.

^eLoop-mediated isothermal amplification (LAMP)=4, nucleic acid sequence-based amplification (NASBA)=3, reverse transcription strand invasion-based amplification (RT-SIBA)=2 studies, Sanger sequencing=1.

Accuracy of DFA. Five studies compared DFA to RT-PCR, of which 4 used a multiplex RT-PCR platform, and 1 study a simplex platform [27–31]. Pooled sensitivity versus multiplex RT-PCR was 83% (95% CI, 66%–93%) across all platforms, and the corresponding pooled specificity was 96.6% (95% CI, 96.2%–97.0%). The only sensitivity estimate limited to adults was the lowest reported at 56% (95% CI, 31%–78%) (Figure 4A and 4B).

Accuracy of RADT. Overall, RADTs had a pooled sensitivity of 64% (95% CI, 50%–75%) compared to a reference standard of RT-PCR based on 14 comparisons from 7 studies. The type of reference standard employed affected RADT sensitivity estimates [32–38]. Overall sensitivity was 77% with multiplex RT-PCR as reference test (n = 7 comparisons) and 50% with singleplex RT-PCR (n = 7). The studies limited to adults reported the lowest sensitivities: BinaxNOW (14%) and RSV ImmuView tests (23%) compared to singleplex RT-PCR [33]. Overall, RADT specificity was 99% (95% CI, 98%–100%) compared to RT-PCR (Figure 4C and 4D).

Accuracy of Viral Culture. We identified 3 studies that assessed viral culture versus a reference standard of RT-PCR, but 1 only included a single positive sample resulting in unstable results. The sensitivity ranged between 49% and 86%. Specificity was 100% in all 3 studies (Supplementary Table 9) [7, 39, 40].

Accuracy of Multiplex Versus Singleplex and Agreement Between RT-PCR Platforms. Six studies evaluated the performance of a multiplex RT-PCR versus a gold standard singleplex platform [27, 41–45]. Pooled sensitivity was 92% (95% CI, 86%–96%) and specificity 99% (95% CI, 98%–100%) (Figure 5A and 5B). The single adults-only study had lowest sensitivity at 81%. Agreement

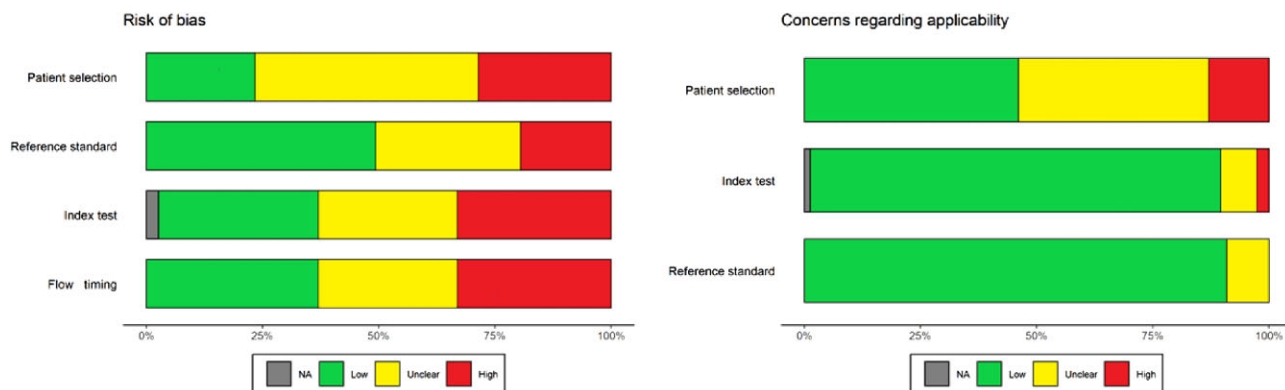


Figure 2. Summary risk of bias assessment of included studies based on the QUADAS-2 tool. Abbreviation: NA, Not applicable.

between RT-PCR platforms was generally high, with a pooled κ value of 0.91 (Supplementary Figure 1).

Sensitivity Analyses

To obtain adult-specific results for the DRRs (section “RSV Detection by Single Sample RT-PCR Versus Combined Testing Approach”), we excluded those studies that involved both adults and children [18, 26]. Compared to the primary analysis, the exclusion of Feikin et al 2013 [18] from the analysis for added paired serology produced similar findings ($DRR_{\text{subanalysis}} = 1.43$ vs $DRR_{\text{full-analysis}} = 1.42$), while exclusion of Kim et al 2011 [26] resulted in a lower DRR for RT-PCR on OPS ($DRR_{\text{subanalysis}} = 1.12$ vs $DRR_{\text{full-analysis}} = 1.28$) (Supplementary Figure 2). We included only 2 studies on adults ≥ 60 years old, hence we could not conduct a subanalysis in this group.

To investigate study quality’s influence on our results, we excluded studies with high risk of bias and mixed adult and children study population from the primary outcome analysis and produced similar results for serology and sputum. For OPS, only 1 study was retained, which showed no increase in RSV detection by (DRR, 1.00; 95% CI, .33–2.99; Supplementary Figure 3A and 3B).

Investigation of Heterogeneity and Subgroup Analysis

We investigated heterogeneity by conducting a stratified analysis of test performance by adult and mixed populations (Figure 4A–4D). While the number of studies in only adults precluded meta-analyses, the overall reported sensitivities of RADT and DFA were higher in mixed adult and pediatric populations than in adults only.

DISCUSSION

To our knowledge, this is the first systematic review that quantifies the underascertainment of RSV infection associated with diagnostic testing and specimen collection approach. RT-PCR

remains the most sensitive testing approach, and NPSs are the most used specimen type for this testing. Despite RT-PCR’s high sensitivity, 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. The use of singleplex rather than multiplex PCR was associated with an additional 7% increase in ascertainment. Furthermore, sensitivity estimates limited to only adults were consistently the lowest reported across several comparisons. Because most RSV incidence studies in adults use only RT-PCR of NPS to detect RSV infection and many use a multiplex RT-PCR platform, our results support a substantial underascertainment of RSV burden in adults, particularly in the hospital setting.

All sample types were found to increase RSV detection when added to NP/nasal swab. Multiple reasons likely account for this increased RSV detection. At the time of presentation to a health care provider or to a hospital, the infection may be predominantly focused in the lower rather than the upper respiratory tract, and thus sputum may be more likely to be positive than NP/nasal swab. Nasal viral shedding in adults begins 1–2 days after symptom onset and starts declining by days 5–6, thus because persons are hospitalized on average 5 to 6 days after illness onset, nasal shedding may be limited at the time of testing [46]. Over half of the populations from included studies were hospitalized, where material captured with NP/nasal swabs can be limited by nasal dryness from nasal oxygen use as well as potentially diuretic administration and dry indoor air due to air handling systems. Sputum is from within the lung and OPS from within the mouth/throat, which would likely be less impacted by such factors [46, 47]. Paired serology can detect recent RSV infections missed by direct viral detection in respiratory samples and may have important epidemiological application. Because of past exposure to RSV, adults can have a rapid and marked increase in humoral immune response allowing for RSV diagnosis based on a 4-fold rise in

Table 2. RSV Detection by Reference Testing and Additional testing

Reference, Author (Year)	Country	Tested Population Description, All Adults Only Unless Otherwise Specified	Subjects With Paired Specimens, No.	Reference Specimen Type, All RT-PCR	RSV Detection Rate by Reference	RSV Detection Rate Using All Specimens	Detection Rate Ratio
Additional testing = paired serology							
Luchsinger (2012) [16]	Chile	Hospitalized with CAP	184	Sputum, NP aspirate, or BAL	10.8	19.6	1.80
Falsey (2019) [14]	Europe/North America	≥50 y with severe COPD/CHF with RS	1111	Nasal swab	2.5	3.8	1.50
Falsey (2002) [7]	USA	Healthy, CHF/COPD, inpatients, and nursing home residents	1112	NP swab	7.8	10.5	1.34
Feikin (2013) [18]	Kenya	Pediatric and adult outpatients with RS	204	NP swab	8.3	11.3	1.36
Korsten (2021) [15]	Belgium, The Netherlands, and England	Community-dwelling adults ≥60 y with RS (not medically attended and outpatients)	1040	NP swab	3.5	5.7	1.64
Zhang (2017) [19]	USA	Hospitalized 18 -64 y with CAP	623	NP swab	2.2	2.9	1.29
Zhang (2017) [19]	USA	Hospitalized ≥65 with CAP	313	NP swab	3.2	4.8	1.50
Additional testing = RT-PCR on oropharyngeal swabs							
Ek (2019) [20]	Sweden	Attending emergency department with RS	98	NP swab	6.1	6.1	1.00
Kim (2011) [26]	Kenya	Pediatric and adult outpatients with RS	2331	NP swab	10.8	14.1	1.30
Lieberman (2009) [21]	Israel	Hospitalized with LRTI	1000	NP swab	2.6	3.0	1.15
Additional testing = RT-PCR on sputum							
Branche (2014) [22]	USA	Hospitalized with RS	965	Nasal and throat swab	5.1	6.5	1.29
Falsey (2019) [14]	Europe/North America	≥50 y with severe COPD/CHF with RS	674	Nasal swab	2.4	4.7	2.00
Jeong (2014) [23]	South Korea	Hospitalized and outpatient with RS	154	NP swab	11.0	18.8	1.71
Additional testing = RT-PCR on nasopharyngeal wash with or without oropharyngeal swabs							
Lieberman (2009) [21]	Israel	Hospitalized with LRTI	1000	NP swab	2.6	2.8	1.08
Lieberman (2009) [21]	Israel	Hospitalized with LRTI	1000	NP swab	2.6	3.1	1.19
Additional testing = RT-PCR on saliva							
To (2017) [25]	China	Hospitalized with RS	159	NP aspirate	11.3	11.3	1.00
To (2019) [24]	China	Hospitalized with RS	212	NP aspirate	11.8	12.7	1.08

Abbreviations: CAP, community-acquired pneumonia; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; LRTI, lower respiratory tract infection; NP, nasopharyngeal; RS, respiratory symptoms; RSV, respiratory syncytial virus; RT-PCR, reverse transcription-polymerase chain reaction.

immunoglobulin G (IgG) antibody levels between the initial acute specimen and convalescent specimen (4–6 weeks later). However, not all with confirmed RSV-infection by PCR or viral culture seroconvert and false-negative serology can also occur due to already elevated IgG level in the acute specimen [46], especially if collected at hospital admission after several days of illness [7, 46].

Because of the limited number of studies reporting on exclusively adult populations, we included estimates from mixed-age populations. We conducted a sensitivity analysis excluding these for the primary end point, but not for other comparisons because the number of adult-only articles were too small. To

address this, we presented results by age group (adults vs mixed/unspecified results) and included age in our figures. The lowest sensitivity estimates across multiple comparisons (ie, DFA, RADT, and singleplex vs multiplex) were those including adults only. Adults are known to have lower viral loads in their respiratory secretions than children [2], likely explaining these lower results. On this basis, the inclusion of mixed populations likely resulted in our estimates of RSV underascertainment being conservative.

We have collated a body of literature that documents that testing single NPS or nasal swab with RT-PCR for RSV diagnosis results in systematic underestimation of the frequency of

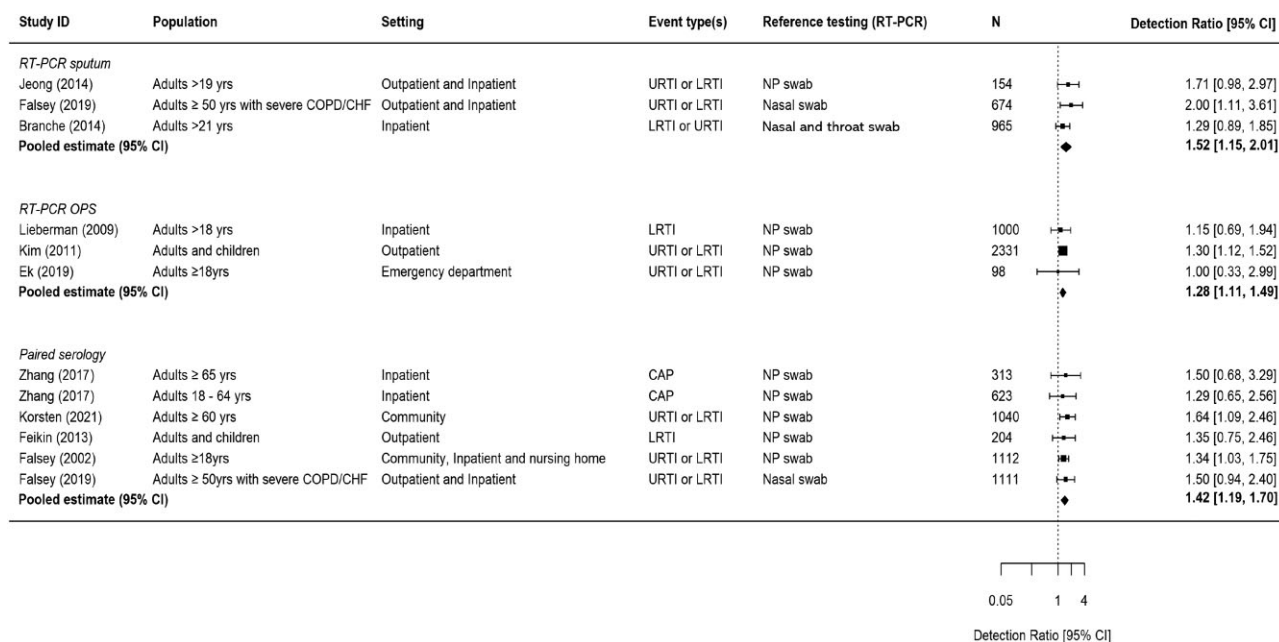


Figure 3. Increase in RSV detection rate due to the addition of another specimen testing to reference RT-PCR of NP swab or nasal swab. Detection ratio >1.0 indicates an increase in RSV detection associated with additional specimen testing. Abbreviations: CAP, community-acquired pneumonia; CHF, chronic heart failure; CI, confidence interval; COPD, chronic obstructive pulmonary disease; LRTI, lower respiratory tract infection; NP, nasopharyngeal; OPS, oropharyngeal swab; RT-PCR, reverse transcription polymerase chain reaction; URTI, upper respiratory tract infection.

RSV infection. While 2 prior reviews have highlighted the poor performance and variability in the performance of RADTs when compared with RT-PCR [4, 42], no reviews have collated information on the additional case yield associated with adding additional specimen types, making our systematic literature review the first to undertake this. Our review further highlights the limited level of agreement between point-of-care molecular tests and conventional RT-PCR (pooled estimated 70% for adult studies only; [Supplementary Figure 1](#)). However, a recent study published after this study's search period found 99.3% overall agreement between Xpert Xpress Flu/RS and an in-house RT-PCR in RSV detection [48].

This has important implications for burden of disease estimates, because nearly all RSV incidence studies among adults depend exclusively on RT-PCR NPS for RSV diagnosis [6]. Recently, a published meta-analysis of US incidence studies included an adjustment factor of 1.5-fold for underascertainment based on diagnostic testing, which increased the pooled prospective incidence estimate of RSV-related hospitalizations among persons 65 years and older from 189 to 282 per 100 000 [49]. Our results suggest this adjustment is conservative because all our collated comparisons were pairwise and did not take into account the additional cases that could result from adding more than 2 specimen types. A prospective study is underway in North America to collect a wide range of specimens for acute respiratory events to describe this overall increase in

sensitivity. While there is some overlap among the additional positives identified by sputum, saliva, and paired serology testing compared to NPS alone, each specimen type contributes some additional unique positives [50].

The primary strengths of this review were a comprehensive systematic literature review that included 3 global databases, inclusion of other potential sources of relevant studies, and broad inclusion criteria. The inclusion of many large studies and multiple comparisons allowed for a broader scope of diagnostic tests comparisons. Furthermore, these results come from a variety of treatment settings (inpatients, outpatients, non-medically attended events from community, nursing home residents), populations (older and younger adults, persons with underlying congestive heart failure, chronic obstructive pulmonary disease, as well as healthy adults), event types (any acute respiratory symptoms, all lower respiratory tract infection, and limited to pneumonia), and geographic regions (Asia, Africa, North and South America, and Europe) supporting the generalizability of these results.

However, there are limitations in this study. First, about one-third (46 of 154) of included studies had fewer than 200 participants and hence small overall numbers of RSV detected. Second, about two-thirds of articles had at least 1 high or unclear risk of bias. This was most often observed in studies comparing diagnostic test performance and less often in studies comparing detection rates using combined specimens.

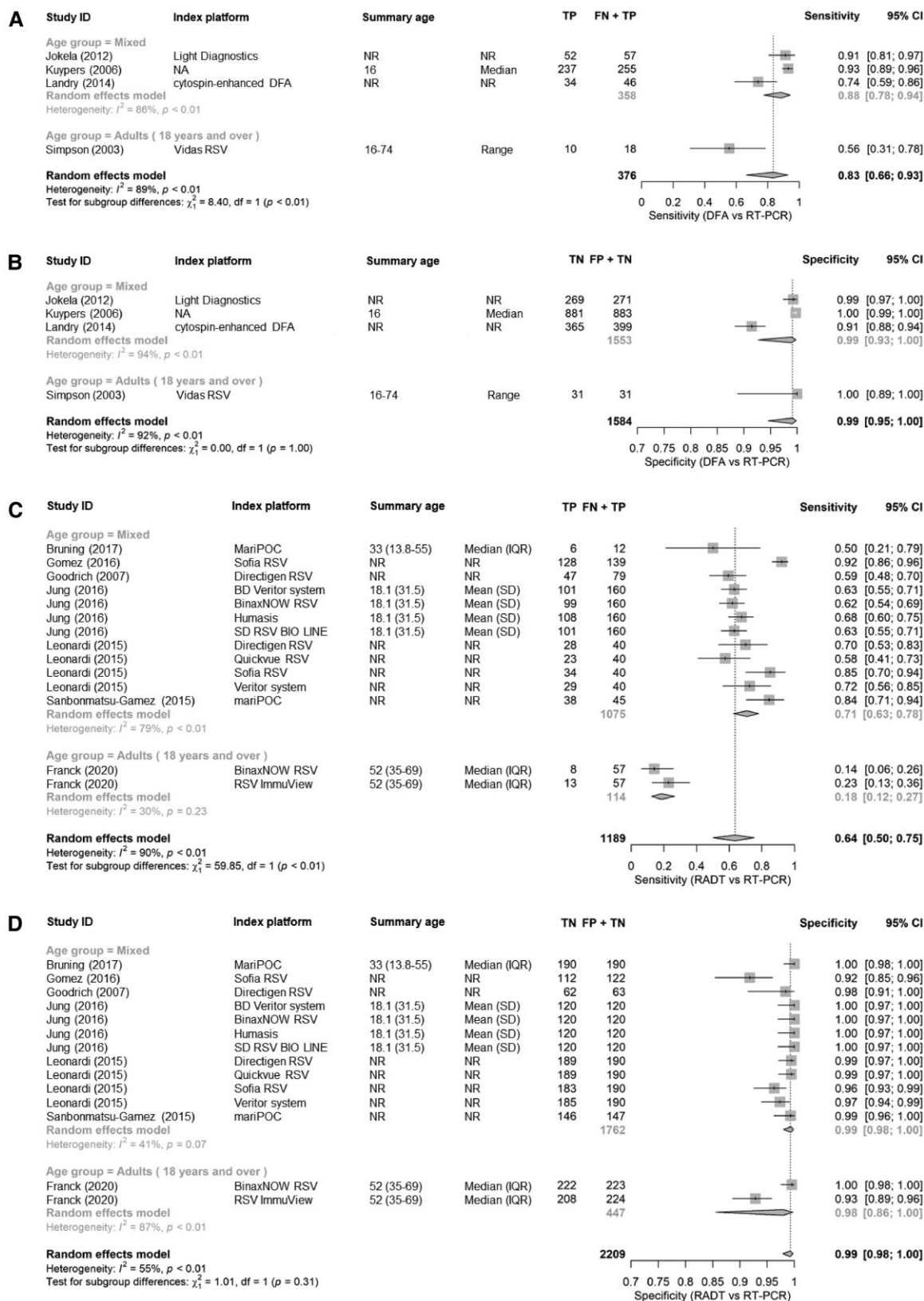


Figure 4. A, Sensitivity of DFA test using RT-PCR as reference test. B, Specificity of DFA test using RT-PCR as reference test. C, Sensitivity of RADT using RT-PCR as reference test. D, Specificity of RADT using RT-PCR as reference test. Abbreviations: CI, confidence interval; DFA, direct fluorescent antibody; FN, false negative; FP, false positive; IQR, interquartile range; NA, Not Applicable; NR, Not Reported; RADT, rapid antigen detection tests; RT-PCR, reverse transcription polymerase chain reaction; TN + FP, all reference negative; TN, true negative; TP, true positive; TP + FN, all reference positive.

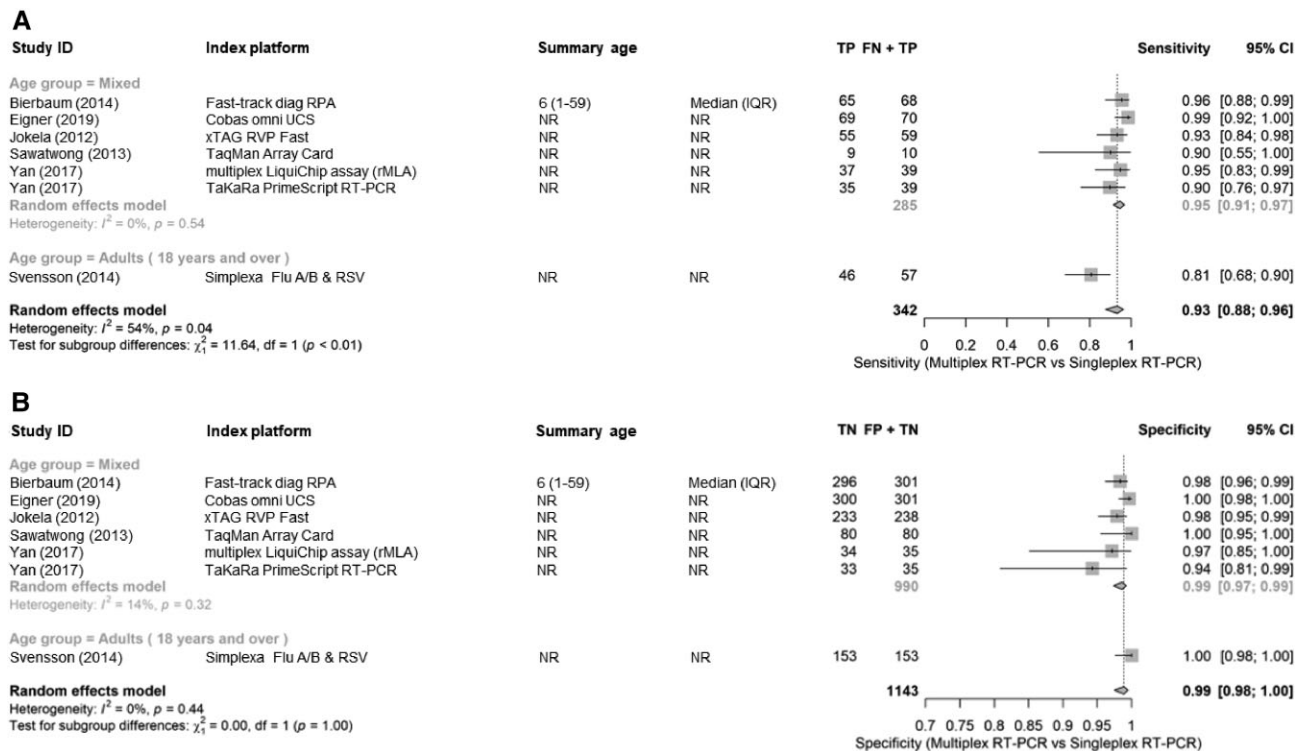


Figure 5. A, Sensitivity of multiplex RT-PCR using singleplex RT-PCR as reference test. B, Specificity of multiplex RT-PCR using singleplex RT-PCR as reference test. Abbreviations: CI, confidence interval; NR, Not Reported; FN, false negative; FP, false positive; IQR, interquartile range; RT-PCR, reverse transcription polymerase chain reaction; TN + FP, all reference negative; TN, true negative; TP, true positive; TP + FN, all reference positive.

Overall, 49 studies had an unclear risk of bias, due to inadequate available information in at least 1 domain. This highlights the gaps in reporting of DTA studies and calls for wider adoption of DTA reporting guidelines. Lastly, as mentioned above, many of the studies included in the analysis of diagnostic test performance provided sparse demographic data or did not provide disaggregated information. This lack of detailed demographic and clinical information precluded the investigation of heterogeneity by subgroup analysis, including one for older adults, likely to benefit most from future vaccines.

CONCLUSIONS

Accurate diagnostic methodologies are critical for determining RSV disease burden, public health impact of preventative interventions (including vaccines), and generating accurate economic models. With multiple RSV vaccine candidates currently under development, the estimation of population-level effects will rely heavily on an accurate diagnosis of infected persons. Our review indicates that, while RT-PCR using NPS is the most sensitive currently available diagnostic methodology, the addition of other testing approaches—including collection of different specimens and potentially use of serology—substantially boosts RSV detection and these results should be considered when estimating disease burden and the subsequent

economic value of RSV immunization of adults. Additional research on synergistic effects of using multiple specimens could further inform this issue and lead to more accurate disease burden estimates and economic models, for example by adding a multiplier to adjust published RSV lower respiratory tract infection incidence estimates.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copy-edited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. P95 was contracted by Pfizer to conduct this study. C. O., L. M. M., S. M., B. M., M. S., N. A., M. R., Z. A.-T., and H. V. are employees of P95. D. C., W. K., J. E. A., B. D. G., M. R., and E. B. are employees of Pfizer and may hold stock or stock options. H. N. declares no conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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