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Citation for published version:
Onwuchekwa, C, Atwell, J, Mora Moreo, L, Menon, S, Machado, B, Siapka, M, Agarwal, N, Rubbrecht, M, Aponte-Torres, Z, Rozenbaum, M, Curcio, D, Nair, H, V Kalina, W, Vroling, H, Gessner, B & Begier, E 2023, 'Pediatric RSV Diagnostic Testing Performance: A Systematic Review and Meta-analysis', *The Journal of Infectious Diseases*. https://doi.org/10.1093/infdis/jiad185

Digital Object Identifier (DOI):
10.1093/infdis/jiad185

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
The Journal of Infectious Diseases

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Download date: 30. Jul. 2023

Pediatric RSV Diagnostic Testing Performance: A Systematic Review and Meta-analysis

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Running title: RSV diagnostic testing in children

Keywords: Respiratory Syncytial Virus Infections; epidemiology; diagnosis; sensitivity and specificity, children

Abstract word count: 200

Main text word count: 3486

Article summary: We investigated whether adding other specimen types to nasopharyngeal/nasal aspirate or swab RT-PCR increased RSV detection in children, as found in adults. RT-PCR was the most sensitive diagnostic test and adding additional specimen types modestly increased RSV detection.

ABSTRACT

Background: Adding additional specimen types (e.g., serology or sputum) to nasopharyngeal swab (NPS) RT-PCR increases respiratory syncytial virus (RSV) detection among adults. We assessed if a similar increase occurs in children and quantified under-ascertainment associated with diagnostic testing.

Methods: We searched databases for studies involving RSV detection in persons <18 years using ≥2 specimen types or tests. We assessed study quality using a validated checklist. We pooled detection rates by specimen and diagnostic tests and quantified performance.

Results: We included 157 studies. Added testing of additional specimens to NP aspirate (NPA), NPS and/or nasal swab (NS) RT-PCR resulted in statistically non-significant increases in RSV detection. Adding paired serology testing increased RSV detection by 10%, NS 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 specificities all ≥98%). Pooled sensitivity of multiplex versus singleplex RT-PCR was 96%.

Conclusions: RT-PCR was the most sensitive pediatric RSV diagnostic test. Adding multiple specimens did not substantially increase RSV detection, but even small proportional increases could result in meaningful changes in burden estimates. The synergistic effect of adding multiple specimens should be evaluated.

Keywords: Respiratory Syncytial Virus Infections; epidemiology; diagnosis; sensitivity and specificity, children

Introduction

Respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory tract infection (LTRI) in children [1]. Globally, an estimated 33 million episodes of RSV LRTI, 3.6 million hospitalizations, and 101,400 deaths occur yearly [2]. RSV is associated with 28% of all childhood acute lower respiratory infection (ALRI) episodes and 13-22% of ALRI mortality in young children [3]. Virtually all children experience RSV within the first two years of life; nearly 40% of RSV-associated hospitalizations in children <5 years of age occur within the first 6 months [2, 4].

No vaccines or RSV-specific treatments are currently available, and care is largely supportive. Because viral identification has limited impact on clinical decision-making, RSV diagnostic testing is not universally performed, and RSV is, therefore, likely underdiagnosed. Diagnostic testing has become more common in recent years and often involves upper respiratory tract specimens, including nasopharyngeal aspirates (NPA), swabs (NPS), nasal swabs (NS), washes (NW), or oropharyngeal swabs (OPS). Testing methodologies include viral culture, rapid antigen detection tests (RADT), immunofluorescence (IF) tests (including direct fluorescent antibody (DFA)), and reverse transcriptase polymerase chain reaction (RT-PCR). Previously, viral culture was considered the gold standard test for RSV, especially in children with high viral shedding; however, it requires several days and special specimen handling, which limits use in clinical settings [5, 6]. DFA tests and RADTs had replaced viral culture for routine clinical testing; more recently, molecular methods have become the gold standard due to their higher sensitivity [6, 7]. Additionally, serum testing can identify recent infections by a ≥4-fold increase in RSV-specific antibody titers between paired acute and convalescent specimens or pre- and post-season specimens. However, up to one-third of children do not seroconvert after infection [6, 8]. The accuracy of each diagnostic test is influenced by specimen type, specimen quality, age of the patient and RSV prevalence [6, 9, 10].

Because disease incidence is a crucial component of decision-making for preventive interventions, it is critical to estimate RSV incidence accurately. In theory, some testing methods and specimen sources may underestimate incidence in children, and even small proportional underestimations could translate to high numbers of missed cases. Our recent review suggested that adding testing of an additional specimen type to nasal/NP swab RT-PCR can increase adult RSV detection by 28-45% [11], depending on the specimen type. However, similar data are needed to assess potential underestimation in other age groups. Therefore, we conducted a systematic review and meta-analysis to quantify RSV under-ascertainment due to different specimens and testing modalities among children. We also summarize the evidence on the diagnostic accuracy of available testing modalities and evaluate factors affecting test performance.

Methods

Protocol and registration

This meta-analysis was part of a larger review on RSV under-ascertainment due to diagnostic approaches in adults and children. The protocol adhered to the Preferred Reporting Items for Systematic Review and Meta-Analyses for Diagnostic Test Accuracy Studies (PRISMA-DTA) and was registered on the PROSPERO database (registration No. CRD42022313209) [12].

Study eligibility criteria

Eligibility criteria were developed using the Population-Intervention-Comparator-Outcomes-Time Frame-Setting (PICOTS) framework (Supplementary Table 1). We identified primary studies reporting on RSV detection in children and young adults (age <18 years), using ≥2 types of specimens or diagnostic tests (index and reference) in the same population. Eligible studies reported the number of RSV cases detected by each testing method or specimen type or presented 2-by-2 tables of test performance.

Information sources and search strategy

We searched Embase, MEDLINE (via PubMed), and Web of Science for publications from 01 January 2000 to 27 December 2021. We also searched non-indexed sources and RSV research networks, such as Respiratory Syncytial virus Consortium in Europe (RESCEU project, https://resc-eu.org/) and Respiratory Syncytial Virus Network (ReSViNET, https://www.resvinet.org/). The bibliographies of selected systematic reviews and meta-analyses were also screened for relevant articles (Supplementary Table 2). Detailed search strategy and search terms are presented in Supplementary Table 3.

Study selection

Retrieved articles were screened based on the eligibility criteria (Supplementary Table 1). Titles and abstracts were first assessed to identify potentially relevant articles. We subsequently conducted independent duplicate full-text screening, with disagreements resolved by a third reviewer. We used DistillerSR to manage the study selection process [13].

Data extraction

We created a structured extraction form on DistillerSR, previously used for a similar review in adults [11]. All extracted data were independently cross-checked to minimize errors.

Definitions for data extraction

The extracted data items are summarized in Supplementary Table 4. We defined children as persons <18 years of age, with the following subgroups: infants (< 12 months), young children (1- < 5 years), and older children (5-18 years). Consistent with the PRISMA-DTA guidance, the index test was defined as the test under evaluation, while the reference test was defined as the comparator test [14]. A true positive (TP) result was positive by both the index and reference test, and a false positive (FP) result was positive by the index test but negative by the

116 reference test. A false negative (FN) result was negative by the index test but positive by the
117 reference test, and a true negative (TN) result was negative by both index and reference tests.

118 Risk of bias and applicability

119 The risk of bias (RoB) assessment of included studies was performed by one reviewer and
120 verified by a second reviewer using the Quality Assessment of Diagnostic Accuracy Studies
121 (QUADAS-2) tool (Supplementary Table 5) [15]. Disagreements were resolved by consensus,
122 and when required, a third reviewer resolved the conflict. We developed an extension to the
123 main QUADAS-2 tool to facilitate bias assessment when comparing specimens using a single
124 test method.

125 Diagnostic accuracy measures and synthesis of results

126 The analytic approach for this review and meta-analysis is detailed elsewhere [11]. Briefly, we
127 describe the included studies and summarize key characteristics of the study population and
128 testing approach (including specimens used and diagnostic method).

129 We compared the RSV detection rate (DR) under combined testing (using multiple specimens
130 or diagnostic tests) and test-specific (reference) conditions, presenting this as detection rate
131 ratios (DRR). For this comparison, RT-PCR using NPA, NPS, or NS was used as the reference
132 testing method in order of sample preference, and all positives were considered true positives.

133 The data from 2-by-2 tables reporting TP, FP, FN, and TN were used to calculate individual
134 studies' test sensitivity and specificity (with 95% confidence intervals). The sensitivity and
135 specificity point estimates were pooled in meta-analyses for RADTs, DFA and viral culture,
136 using RT-PCR as a common reference. All meta-analyses were performed under random-
137 effects assumptions [5, 16] and were restricted to analyses with ≥3 studies. Each index-
138 reference comparison was considered a separate experiment, presented separately, and

139 contributed to the meta-analysis estimates. All analyses were performed in R version 4.2.0,
140 using the meta4diag and metafor packages [17].

141 Subgroup and sensitivity analyses
142 In the meta-analysis of test performance, we stratified findings by the type of RT-PCR used as
143 the reference test, either singleplex or multiplex. We also stratified test performance by the
144 following pediatric age categories for which estimates were available: infants, all under 5 years
145 (without further disaggregation), and all under 18 years (without further disaggregation).
146 Estimates limited to older children were not available.

147 We also assessed the impact on the pooled results of study quality, based on the QUADAS-2
148 assessment, by conducting separate meta-analyses, including only studies with a low risk of
149 bias.

150 **Results**

151 Study selection process
152 Our search identified 8066 unique references, of which 157 studies were included in the review
153 (Figure 1 and Supplementary Table 6). The most common reasons for exclusion at full-text
154 level were studies conducted in adults or not reporting estimates for those aged <18 years
155 separately, studies conducted before 2000, and those not reporting on RSV detection by
156 multiple methods.

157 Study characteristics
158 Supplementary Table 7 provides detailed descriptions of included studies. Over 60% of studies
159 were conducted in the Americas and Europe, and 93% used a cross-sectional design (Table 1).
160 Most studies included hospitalized children (67%), and the most common clinical presentation
161 was LRTI, including bronchiolitis, pneumonia and non-specified acute respiratory infections
162 (67.5%).

163 NPAs were collected in 85 studies (54.1%), while 53 (33.8%) used NPS. Multiplex and
164 singleplex RT-PCR were evaluated in 86 (54.8%) and 50 (31.8%) studies, respectively, while
165 RADTs were evaluated in 56 studies (35.7%). Among included studies, 55 (35.0%) reported
166 on children 0-5 years of age, 62 (39.5%) included children 0-18 years, and 6 (3.8%) were
167 conducted on infants 0-1 year. Thirty-four studies (21.7%) did not clearly specify the age range
168 of children included in the study.

169 Risk of bias and applicability

170 Supplementary Table 8 provides a detailed assessment of the risk of bias and applicability at
171 domain level. Overall, 20 studies (12.3%) had a low risk of bias assessment in all domains,
172 while 37 (22.7%) and 99 studies (60.7%) had a high or unclear risk of bias in at least one
173 domain, respectively. Figure 2 provides a graphical summary of the risk of bias assessment at
174 domain level for all eligible studies. In the patient selection domain, nearly half of the studies
175 had an unclear risk of bias assessment, and 18 studies had a high risk of bias. Common reasons
176 for unclear or high risk of bias in this domain were unclear patient population and study designs
177 that could be interpreted as case-control designs. Risk of bias concerns for the index and
178 reference tests were often due to failure to report whether test operators were blinded or if
179 previously characterized stored specimens were used. Risk of bias in the index test domain was
180 not performed in 10% of studies where sample types were compared using the same test
181 (considered reference, e.g., RT-PCR of two different specimen types). Risk of bias in the
182 domain of flow and timing was assessed as unclear in 50 studies (32%), often because it was
183 not explicitly reported that both the index and reference tests were conducted in the same period
184 of illness and whether all included patients had the same reference test (e.g., studies applying
185 a composite reference). Less than 10% of studies had high/unclear applicability concerns in
186 any of the 3 relevant domains.

187 Results of individual studies

188 Supplementary Table 9 provides the number of RSV infections detected by each diagnostic test
189 and specimen type combination obtained in each study. It also provides overall RSV detection
190 by combination testing and test-specific detection rates. Supplementary Table 10 displays the
191 diagnostic performance, in terms of sensitivity and specificity, of included studies stratified by
192 test type.

193 Synthesis of results

194 Increase detection of RSV by combined specimen testing

195 Testing of multiple respiratory specimens by any method (i.e., RADT, DFA, RT-PCR or paired
196 serology) resulted in a small overall increase in RSV detection (all non-statistically significant
197 trends) for the specimen types examined (Table 2 and Supplementary Figure 1). Using RT-
198 PCR on NPA/NPS or paired specimens, additional testing of OPS to NPS/NPA increased RSV
199 detection by 5% (DRR 1.05, 95% CI 0.96-1.14). Adding testing of NS to NPA/NPS increased
200 detection by 8% (DRR 1.08, 95% CI 0.94-1.25), while adding NPS to NPA increased RSV
201 detection by 1% (DRR 1.01, 95% CI 0.94-1.08) (Figure 3). Excluding 4 studies with high or
202 unclear risk of bias did not change these results substantially (Supplementary Figure 2) [18-
203 21]. Adding paired serology testing to RT-PCR on NPS resulted in a 10% increase in RSV
204 detection in one study (DRR 1.10, 95% CI 0.95-1.27) [22]. Stratified data allowed calculation
205 of detection ratios by age group for added paired serology: 0 – 6 months: DRR 1.02 (95% CI
206 0.76-1.36), 7 months-2 years: DRR 1.12 (95% CI 0.94-1.33), 3-5 years: DRR 1.04 (95% CI
207 0.73 – 1.48), 6-17 years: DRR 1.29 (95% CI 0.65 – 2.54). Adding sputum RT-PCR increased
208 RSV detection by 11.7% compared to OPS RT-PCR alone in one study (124/440 versus
209 111/440) [23]. Adding RT-PCR of BAL increased detection by 26.7% compared to OPS-PCR
210 alone in one study (19/76 versus 15/76) [24].

211 Performance of RADT versus RT-PCR

212 We included 21 studies performing 28 comparisons of RADT as index tests compared to RT-
213 PCR with a pooled sensitivity of 75% (95% CI 69-80%) with a range of 26-100% (Figure 4).
214 Stratified by population age, the pooled sensitivity was 63% in studies limited to children 0-5
215 years (95% CI 49-76%) and 78% in studies including children up to 18 years (95% CI 74-
216 82%). When stratified by the type of RT-PCR test, the pooled sensitivity of RADT against
217 singleplex RT-PCR was 70% (95% CI 59-79%) and 76% against multiplex RT-PCR (95% CI
218 71-81%). Overall, the specificity of RADT was 97% (95% CI 95-98%) with little change when
219 stratified by age groups or RT-PCR type.

220 Performance of DFA versus RT-PCR

221 The pooled sensitivity of DFA against RT-PCR was 87% (95% CI 78-93%) and ranged
222 between 72-96%, with similar results obtained with singleplex and multiplex RT-PCR
223 references (Figure 5). Stratified by age groups, pooled sensitivity was 82% in studies limited
224 to children 0-5 years (95% CI 76-87%) and 88% in studies reporting on all children 0-18 years
225 (95% CI 73-95%). The pooled specificity of DFA was 99% (95% CI 97-100%), with similar
226 results obtained when stratified by age groups and RT-PCR types.

227 Performance of viral culture versus RT-PCR

228 Viral culture had a pooled sensitivity of 76% compared to RT-PCR (95% CI 71-81%) with a
229 range of 75-100% and a pooled specificity of 100% (95% CI 99-100%). It was not possible to
230 assess the performance of viral culture by age or type of RT-PCR reference owing to the small
231 number of studies included in the analysis (Figure 5).

232 Performance of multiplex versus singleplex RT-PCR and agreement between multiple
233 platforms
234 Most of the commonly used multiplex platforms performed well against singleplex RT-PCR.
235 The pooled sensitivity of multiplex RT-PCR was 96% (95% CI 92-98%), while pooled
236 specificity was 100% (95% CI 98-100%) (Supplementary Figure 3). The performance of
237 multiplex against singleplex RT-PCR showed little variation by subject age range.

238 **Discussion**

239 To our knowledge, this is the first systematic review and meta-analysis to report on both
240 specimen and diagnostic test performance in children compared to common reference methods.
241 Overall, we found that additional testing of other specimen types to NPS/NPA RT-PCR did not
242 substantially increase RSV detection in pairwise comparisons (1-10% average increase
243 depending on the additional specimen type). However, given the higher disease burden among
244 children, this could meaningfully increase incidence estimates. Variation within individual
245 studies was also modest, with a maximum reported increase in detection of 24% associated
246 with additional RT-PCR testing of nasal swabs. While RT-PCR was the most sensitive
247 diagnostic method, DFA was comparatively more sensitive than RADT and viral culture in
248 detecting RSV infections. All 3 diagnostic tests had specificities above 97%. Similar to prior
249 findings in adults [11], using multiplex PCR rather than singleplex resulted in a small
250 decrement in sensitivity (4%).

251 Our findings suggest that in children presenting with LRTI, routine testing by RT-PCR using
252 a single NPA or NPS specimen is unlikely to miss RSV cases or only a relatively small
253 proportion. And, while we found that additional testing of BAL or sputum may increase
254 detection more substantially, the small number of studies we found means we cannot draw
255 conclusions on the value of adding lower respiratory specimens. This contrasts with results
256 obtained from our review in adults, where adding an additional specimen to NPS increased

257 RSV detection by 28–45%, depending on specimen type [11]. This difference between children
258 and adults may be due to several reasons. Firstly, our analysis of RSV detection in adults used
259 NPS as a reference specimen type because this was most commonly used in adults and is less
260 sensitive than NPA for RSV detection. Secondly, most studies in adults collected additional
261 lower respiratory tract specimens, such as sputum, which may produce higher viral copies for
262 detection [19, 25, 26]. Thirdly, on average, children exhibit higher levels and more extended
263 periods of RSV shedding than adults [27-30], have a smaller upper airway space, and may
264 present earlier during illness. These factors may increase the likelihood of finding a positive
265 result from a single upper airway specimen.

266 We identified one study that investigated the added RSV detection using paired serology,
267 resulting in a 10% increase in RSV detection in children with community-acquired pneumonia
268 [22]. As an indicator of recent infection, a 4-fold increase in acute-convalescence serology
269 titers has been shown to increase RSV detection in adults [31]. However, the interpretation of
270 serological titers may be complicated by a lower likelihood of seroconversion in children [8,
271 32]. This may be partly attributed to IgG maternal antibodies in infants, producing high
272 baseline antibody levels and effectively masking the immune response during infection [33].

273 Compared to specimen type and number, we observed a greater variation by diagnostic method.
274 Compared to RT-PCR, DFA was most sensitive (87%), followed by viral culture (76%) and
275 RADT (75%), with the latter value consistent with other pediatric studies and higher than
276 reported in adults [5]. Our results document a higher sensitivity of these testing modalities in
277 children compared to our results in adults [11], which may be linked to higher viral shedding
278 in children, as prior infection in adults results in a greater immune response and lower shedding.
279 Also, NPA is used more frequently for RSV diagnosis in children, which may increase the
280 sensitivity of RADTs, owing to the higher viral yield of NPA compared to NPS [19].
281 Regardless, adjusting pediatric RSV incidence estimates upwards by 13-26% may be

reasonable depending on the testing method used when studies use RADT, DFA or viral culture instead of RT-PCR.

Strengths and limitations

One of the key strengths of our review was the added search of relevant grey literature sources and RSV networks (Supplementary table 2). This addition resulted in a large number of relevant studies representing global data. We also included a range of clinical settings, clinical presentations, and multiple specimen and testing combinations, giving the results of this review wider utility.

A limitation of our review is that 60% of studies had an overall high/unclear risk of bias. Most of the included studies also did not include sufficient demographic data or information about specimen storage and handling; hence the effect of these factors could not be evaluated within this meta-analysis. Appropriate reporting of DTA studies following the STARD (Standards for Reporting of Diagnostic Accuracy) guidance will mitigate this limitation [34]. Furthermore, prominent reasons for the attribution of a high risk of bias included the use of a case control design, which may inadvertently increase participants who are the "sickest of the sick" or the "wellest of the well"[35], thereby inflating diagnostic accuracy [36]. In addition, a bias could have been introduced when a non-automated index test was interpreted with prior knowledge of the reference test [36].

Our comparison of diagnostic test performance used RT-PCR as a common reference. A limitation of this approach is that the true accuracy of RT-PCR remains a relatively unknown quantity, and implicit assumptions of its accuracy are made. However, our analysis shows that RT-PCR consistently detects >95% of pediatric RSV infections identified by all testing modalities (Supplementary Table 9), with DFA, RADT and viral culture detecting the other

305 5% missed by RT-PCR. These findings support our approach of using RT-PCR as a "gold
306 standard" reference for test accuracy evaluations.

307 Our review also did not stratify the analysis by retrospective and prospective studies because
308 few studies were adequately similar to analyze test performance. We also did not capture the
309 duration of specimen storage, as this may also influence diagnostic test performance.

310 **Conclusions**

311 Compared to adults, where we observed a substantial statistically significant increase in RSV
312 detection, the magnitude of the added benefit in a pediatric population was more modest,
313 especially regarding adding additional specimen types. Given the modest levels of increased
314 detection in published pairwise comparisons, assessing the combined effects of adding multiple
315 specimen types would likely be the most efficient way to study this issue further. In adults,
316 each added specimen type identifies some unique additional cases creating a synergistic effect
317 in the cumulative increase in RSV detection [37]. Further, including saliva as an additional
318 specimen type in such studies would be warranted. While we did not find RSV studies
319 examining saliva, it has recently been shown to be a high-yield specimen type in adults [37]
320 and for SARS-CoV-2 detection [38]. Such studies could confirm the appropriate level of
321 adjustment for epidemiologic data when a single NPA or NPS specimen type is used in disease
322 incidence studies.

323 Under-ascertainment was documented for studies that used diagnostic methods other than RT-
324 PCR. This finding might be incorporated into RSV incidence estimates, future estimates of
325 vaccine effectiveness, and population-level impact once RSV vaccines become available. RT-
326 PCR remains the most sensitive test for RSV, having the advantages of rapid turn-around time
327 and lower running costs. Although RADTs may offer an acceptable alternative in some
328 settings. Further studies are needed to understand the combined effect of specimen and

329 diagnostic test choice, specimen handling and other external factors on RSV detection. Even
330 small proportional increases in RSV detection, when applied to large numbers of RSV
331 infections, could result in meaningful changes in burden estimates.

332

333 **Footnotes**

334 **Conflict of Interest,**

335 P95 was contracted by Pfizer to conduct this study. Neha Agarwal, Zuleika Aponte-Torres,
336 Belen Machado, Sonia Menon, Laura Mora Moreo, Chukwuemeka Onwuchekwa, Michelle
337 Rubbrecht, Mariana Siapka, Hilde Vroling are employees of P95, which received funding from
338 Pfizer in connection with the development of this manuscript. Harish Nair received grants from
339 Innovative Medicine Initiative, National Institute of Health and Care Research, Bill and
340 Melinda Gates Foundation, WHO and Pfizer, and consultancy or honoraria and speaker fees
341 from Sanofi, Merck, Novavax, ReViral and GSK (all paid to institution). Jessica E. Atwell,
342 Elizabeth Beiger, Daniel Curcio, Bradford D. Gessner, Warren Kalina, and Mark Rozenbaum
343 are employees of Pfizer and may hold stock or stock options.

344 **Funding**

345 This work was supported by Pfizer Inc., USA.

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470 **Figure Legends**

471 **Figure 1.** PRISMA flow chart showing the study selection process

472 **Figure 2.** Summary risk of bias assessment of included studies based on the QUADAS-2 tool

473 **Figure 3.** Increase in RSV detection rate due to the addition of another specimen testing to
474 reference RT-PCR of Nasopharyngeal aspirate/swab or Nasal swab

475 (Abbreviations: ARI= acute respiratory infection, URTI= upper respiratory tract infection,
476 LRTI= lower respiratory tract infection, NR= not reported, CI= confidence interval)

477 **Figure 4a.** Sensitivity of Rapid antigen detection tests (RADT) test using reverse
478 transcription polymerase chain reaction (RT-PCR) as reference test

479 (Abbreviations: TP= true positive, FN= false negative, TP + FN= all reference positives)

480 **Figure 4b.** Specificity of Rapid antigen detection tests (RADT) test using reverse
481 transcription polymerase chain reaction (RT-PCR) as reference test

482 (Abbreviations: TN= true negative, FP= false positive, TN + FP= all reference negative)

483 **Figure 5a.** Sensitivity of Direct fluorescent antibody test (DFA) using reverse transcription
484 polymerase chain reaction (RT-PCR) as reference test

485 (Abbreviations: TP= true positive, FN= false negative, TP + FN= all reference positives)

486 **Figure 5b.** Specificity of Direct fluorescent antibody test (DFA) using reverse transcription
487 polymerase chain reaction (RT-PCR) as reference test

488 (Abbreviations: TN= true negative, FP= false positive, TN + FP= all reference negative)

489 **Figure 5c.** Sensitivity of Viral culture using reverse transcription polymerase chain reaction
490 (RT-PCR) as reference test

491 (Abbreviations: TP= true positive, FN= false negative, TP + FN= all reference positives)

492 **Figure 5d.** Specificity of Viral culture using reverse transcription polymerase chain reaction
493 (RT-PCR) as reference test

494 (Abbreviations: TN= true negative, FP= false positive, TN + FP= all reference negative)

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