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Meta-Analysis of the Diagnostic Efficacy of the Luminex xTAG Respiratory Viral Panel FAST v2 Assay for Respiratory Viral Infections

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Purpose: Acute respiratory viral infections pose significant morbidity and mortality, making it essential to diagnose respiratory viral infections rapidly. In this study, the diagnostic efficacy of the Luminex xTAG Respiratory Virus Panel (RVP) FAST v2 test was evaluated on respiratory viral infections.

Materials and Methods: Information was retrieved from electronic databases, including Embase, Web of Science, PubMed, and Cochrane Library, for systematic review. Studies that fulfilled predefined inclusion criteria were included. After the extraction of information, statistical software was utilized for quality evaluation, data analysis, and assessment of publication bias.

Results: Eighty groups in fourfold tables from nine articles were included to perform statistical analyses. Therein, the mean specificity and mean sensitivity of Luminex xTAG RVP FAST v2 test for the detection of respiratory viral infections were 0.99 (0.98–0.99) and 0.88 (0.87–0.90), respectively. Additionally, the negative and positive likelihood ratios were 0.14 (0.11–0.19) and 87.42 (61.88–123.50), respectively. Moreover, the diagnostic odds ratio and area under the curve of summary receiver operating characteristic were 714.80 and 0.9886, respectively.

Conclusion: The Luminex xTAG RVP FAST v2 test could be a reliable and rapid diagnostic method for multiple respiratory viral infections.

Key Words: Luminex, Respiratory Virus Panel FAST assay v2, respiratory viruses, xTAG RVP FAST v2

INTRODUCTION

Respiratory viruses are the most common causative agents of disease in humans, with significant influence on morbidity and mortality worldwide, mainly in children.[1-3] A broad spectrum of diseases is caused by common respiratory viruses, including influenza A virus (IFVA), influenza B virus (IFVB), adenovirus (AdV), parainfluenza virus (PIV), respiratory syncytial virus (RSV), human coronavirus (HCoV), human metapneumovirus (HMPV), human bocavirus (HBoV), and human enterovirus/human rhinovirus (HEV/HRV), etc.[4-11] The common symptoms of respiratory viral infections include fever, cough, sore throat, etc.[12] Previous studies reported that 15%–38% of respiratory viral infections develop into acute lower respiratory infections, typically appearing with severe signs and symptoms of croup, wheezing, pneumonia, high fever, or bronchiolitis.[13] In severe cases, multiple organ dysfunction syndromes may ensue, which

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can lead to death. A such, the mortality rate of respiratory viral infections is noteworthy.[14]

At present, traditional means of rapid detection are generally used to detect respiratory viruses. Cell culture-based virus isolation is generally regarded as the "gold standard" for the detection and diagnosis of viruses.[15] Among rapid detection methods, nucleic acid amplification test and direct fluorescent antibody test have recently been developed as new tools for clinical diagnosis.[5,7,16-18]

The Luminex xTAG Respiratory Viral Panel (RVP) FAST v2 assay (Luminex Molecular Diagnostics, Toronto, ON, Canada) is a qualitative multiplex molecular diagnostic assay for simultaneous detection of 19 viral types and subtypes within 2 hours in a single reaction, including IFVA, IFVB, AdV, PIV, RSV, HCoV, HMPV, HBoV, and HEV/HRV.[9,15,19,20] The xTAG and xMAP® Technology platforms offer a streamlined, quality, high-performance workflow for rapid screening and detection of multiple respiratory viruses.[16,17] Research has shown that the Luminex xTAG RVP FAST v2 is more cost-effective than standard viral direct fluorescent antibody and culture[21] and that its positive rate of detection for major respiratory viruses (RSV, AdV, influenza viruses, and PIV) was higher than that of conventional viral isolation and direct immunofluorescence methods, making it of potential use for diagnosing respiratory viral infections.[19]

This study aimed to assess the accuracy of xTAG RVP FAST v2 for the detection of respiratory viral infections via a systematic review of the literature.

MATERIALS AND METHODS

Literature search

We performed a comprehensive search of four databases (Embase, Web of Science, PubMed, and Cochrane Library) to identify eligible studies until August 14, 2021. In those databases, we searched for articles using the following strategy: ("xTAG RVP FAST v2" OR "xTAG RVP assay" OR "xTAG respiratory viral panel" OR "xTAG respiratory virus panel" OR "xTAG respiratory viral panel assay" OR "xTAG respiratory virus panel assay").

Inclusion and exclusion criteria

Before screening the publications, we adopted inclusion and exclusion criteria in advance. Inclusion criteria were as follows: 1) the purpose and contents of studies should be relevant to xTAG RVP FAST v2 and respiratory viral infections; 2) diagnosis-based studies; 3) original research; and 4) data could be fully extracted. Studies were excluded if they were 1) duplicates, letters, oral presentations, conference abstracts, under publication articles, or case reports; 2) lacking a reference standard; 3) not appropriate for extracting data; or 4) irrelevant studies.

Data extraction

Extracted data included the first author's full name, research design, year of publication, reference standard, type of samples, source of patients or samples, type of virus, true-negative, true-positive, false-negative, false-positive, and total number of samples.

Quality assessment

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool, which is designed for systematic reviews of diagnostic studies, was utilized. QUADAS-2 covered four aspects: index test, reference standard, patient selection, and timing and flow. For this purpose, two researchers assessed the same article and then discussed it to achieve a consistent result. If a consensus could not be reached, a third researcher would intervene and participate in the decision-making process.

Statistical analysis

In total, three relevant statistical software packages were used to perform statistical analysis in our study. First, we performed statistical analysis of the extracted fourfold table data and then divided the fourfold table data into subgroups for further analysis according to the virus samples. We presented results on mean specificity, sensitivity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) in forest plots, and summary receiver operating characteristic (SROC) curves with Meta DiSc 1.4 software (Ramony Cajal Hospital, Madrid, Spain). Subgroup analysis was performed according to the type of virus. A bivariate boxplot was constructed to analyze outlier results. In addition, Stata 12.0 software (StataCorp LLC, College Station, TX, USA) was utilized to identify potential publication bias. All evaluations of methodological quality were performed using Review Manager 5.3 software (The Nordic Cochrane Centre, Copenhagen, Denmark).

RESULTS

Retrieval results

Herein, 285 articles in total were collected, all of which were retrieved systematically from electronic databases. After excluding 142 duplicates, 143 articles remained. After reading the title/abstract and checking the article type, we screened 103 articles. After further reviewing the full-text, nine qualified studies were selected according to the exclusion and inclusion criteria.[22-30] The specific reasons for exclusion as listed in Supplementary Fig. 1 (only online).[31]

Characteristics of eligible studies

Among the nine studies, both prospective and retrospective studies were included, and we extracted 80 four-grid tables from these studies. These studies reported the results of xTAG RVP FAST v2 for detecting multiple viruses (four-lattice tables are shown in Table 1). The patient samples in the included articles were from Canada, France, Finland, Italy, and Vietnam,

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Table 1. Characteristics of the Included Studies (n=9)

Author	Study design	Country	Sample	Ref. method	Virus subgroups	TP	FP	FN	TN	Total
Gadsby, et al[22]	Retrospective	UK	BAL & NPS	RT-PCR	AdV	13	8	0	265	286
					EV/RV	60	17	12	207	296
					hBoV	12	7	0	257	276
					hCoV-HKU1	1	0	0	285	286
					hCoV-NL63	2	0	0	284	286
					hCoV-OC43	7	0	2	277	286
					hMPV	8	1	0	277	286
					IFVA	11	0	5	270	286
					IFVB	4	0	0	282	286
					PIV-3	1	2	0	283	286
					PIV-4	1	0	0	285	286
					RSV	36	2	2	246	286
Pabbaraju, et al[23]	Retrospective	Canada	BAL & NPS	RVP v1	AdV	27	1	1	305	334
					EV/RV	34	6	0	294	334
					hCoV	52	3	3	276	334
					hMPV	28	0	2	304	334
					IFVA	61	0	2	271	334
					IFVB	19	0	27	288	334
					PIV	46	0	5	283	334
					RSV	36	1	1	296	334
Takao, et al[24]	Retrospective	Japan	NPA	NAT	AdV	5	0	0	62	67
					hBoV	5	0	1	61	67
					hMPV	4	0	0	63	67
					IFVA-H1	6	0	0	61	67
					IFVA-H3	6	0	0	61	67
					IFVB	1	0	0	66	67
					Novel IFVA-H1	15	0	2	50	67
					RSV	10	1	0	56	67
Jokela, et al[25]	Prospective	Finland	BAL & NPS	DFA	AdV	4	5	1	318	328
					IFVA	3	1	0	324	328
					IFVB	20	0	2	306	328
					PIV-1	2	2	1	323	328
					PIV-2	2	2	0	324	328
					PIV-3	17	0	1	310	328
					RSV	52	5	2	269	328
				RT-PCR	EV/RV	55	18	7	214	294
					hMPV	11	0	1	282	294
					IFVA	28	0	9	8	45
					RSV	55	2	4	233	294
Mansuy, et al[26]	Prospective	France	NPS	RT-PCR	EV/RV	118	44	18	410	590
					IFVA-H1N1	164	11	18	397	590
Salez, et al[27]	Retrospective	France	NPS	RT-PCR	EV/RV	28	2	6	130	166
					hBoV	1	0	4	161	166
					hCoV	9	0	4	153	166
					hCoV-229E	1	0	0	165	166
					hCoV-HKU1	1	0	3	162	166
					hCoV-NL63	4	0	0	162	166
					hCoV-OC43	3	0	1	162	166
					hMPV	6	0	1	159	166
					IFVA	15	4	2	145	166

Table 1. Characteristics of the Included Studies (n=9) (continued)

Author	Study design	Country	Sample	Ref. method	Virus subgroups	TP	FP	FN	TN	Total
					IFVA-H1N1	15	4	1	146	166
					IFVB	4	0	5	157	166
					PIV	2	0	1	163	166
					RSV	4	0	2	160	166
Esposito, et al[28]	Prospective	Italy	NPS	RT-PCR	AdV	5	3	9	125	142
					EV/RV	14	5	1	122	142
					hMPV	6	2	4	130	142
					RSV	52	7	3	80	142
Luchsinger, et al[29]	Prospective	Chile	NPS & NPA	rtRT PCR	AdV	2	0	1	176	179
					hCoV	1	1	0	177	179
					hMPV	3	1	0	175	179
					IFVA/IFVB	12	2	0	165	179
					PIV	3	1	0	175	179
					RSV	5	0	1	173	179
					RV	42	2	0	135	179
Thi, et al[30]	Retrospective	Vietnam	Nose and throat swabs	RT-PCR	AdV	19	11	2	410	442
					EV/RV	89	69	1	283	442
					hBoV	33	2	10	397	442
					hCoV	16	4	2	420	442
					hMPV	20	8	1	413	442
					IFVA-H1N1	9	1	0	432	442
					IFVA-H3N2	10	0	0	432	442
					IFVA-matrix	3	0	0	439	442
					IFVB	9	0	1	432	442
					PIV-1	3	3	0	436	442
					PIV-2	3	0	0	439	442
					PIV-3	32	7	3	400	442
					PIV-4	5	7	0	430	442
					RSV-A	9	0	1	432	442
					RSV-B	10	0	3	429	442

Ref. method, reference standard method; TP, true positive; FP, false positive; FN, false negative; TN, true negative; BAL, bronchoalveolar lavage; NPS, nasopharyngeal swabs; DFA, direct fluorescent assay; NPA, nasopharyngeal aspirates; RT-PCR, reverse transcription-polymerase chain reaction; rtRT PCR, real time reverse transcriptase polymerase chain reaction; NAT, nucleic acid amplification test; RVP V1, respiratory viral panel version 1; IFVA, influenza virus A; IFVB, influenza virus B; AdV, adenovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hBoV, human bocavirus; hCoV, human coronavirus; hMPV, human metapneumovirus; EV/RV, enteroviruses/rhinoviruses; RV, rhinovirus.

respectively.

Overall data analysis

The forest plots analyzed by using Meta-DiSc statistical software are illustrated in Figs. 1 and 2. The mean specificity and mean sensitivity with 95% confidence interval (CI) of xTAG RVP FAST v2 for detecting respiratory viruses were 0.99 (0.98–0.99) and 0.88 (0.87–0.90), respectively. Additionally, NLR, PLR, and DOR values were 0.14 (0.11–0.19), 87.42 (61.88–123.50), and 714.80 (484.79–1053.94), respectively. In the SROC curve, the value of the area under the curve (AUC) was 0.9886 in Fig. 3A.

Subgroup analyses

Different respiratory viruses were grouped into subgroups. We combined data on the same viruses from different studies for further analysis: an individual study could have comprised more than one subgroup due to different subtypes of a virus. Subgroup analyses revealed differences in mean sensitivity and specificity between the subgroups (Table 2). AUC values under SROC curves were close to 1 for all virus-based groups. Reverse transcription-polymerase chain reaction (RT-PCR) exhibited a mean sensitivity of 0.88 (0.86–0.90) and a specificity of 0.98 (0.98–0.98), sinker to the overall results.

Quality assessment

Quality evaluation in each study was carried out independently by two researchers using the QUADAS-2 tool in Review Manager 5.3 software. Fig. 3B shows the assessment results of the risk

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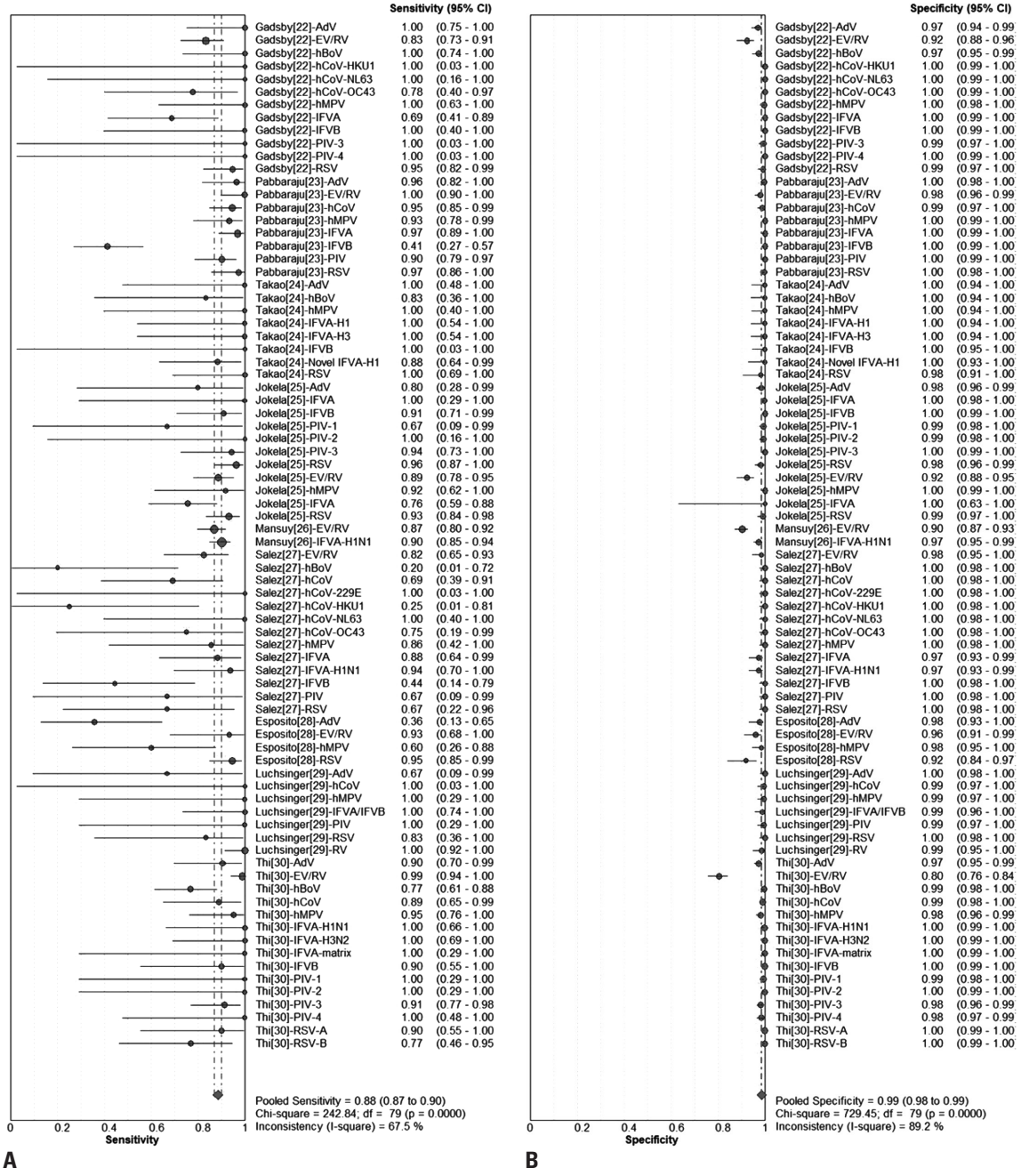

Fig. 1. Forest plots for xTAG RVP FAST V2 in detecting respiratory viruses. (A) Forest plot of sensitivity. (B) Forest plot of specificity.

of bias and applicability concerns in these articles. Finally, we concluded that eight of the studies posed little concern in regards to applicability and bias, except for Jokela's.[25] Bivariate boxplots revealed that several sets of data were out of the circles in Fig. 3C.

Publication bias

For a visualized inspection of publication bias, robustness of the meta-analysis to publication bias was assessed using Deek's funnel plot asymmetry test. The evaluation results indicated low publication bias (p>0.05) (Fig. 3D). Deek's funnel plot asymmetry testing for individual viruses was also performed as shown in Table 2.

DISCUSSION

Precise and rapid recording of respiratory specimens may have a significant influence on disease progression.[32] Accordingly, we evaluated the efficacy of xTAG RVP FAST v2 to detect respiratory viruses. Herein, the mean specificity and sensitivity of xTAG RVP FAST v2 were 0.99 and 0.88, respectively. The NLR, PLR,

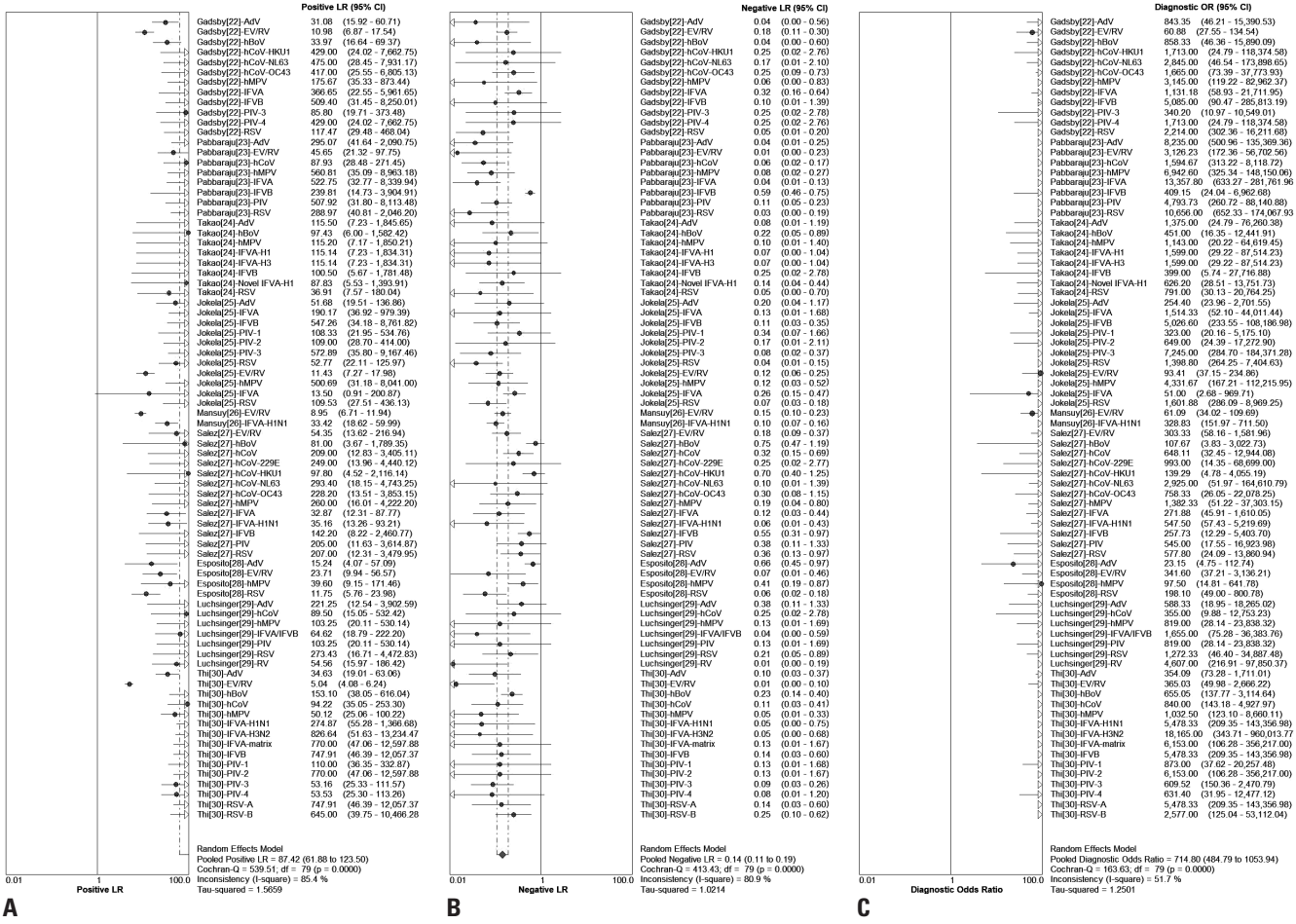

Fig. 2. Forest plots for xTAG RVP FAST v2 in detecting respiratory viruses. (A) Forest plot of positive likelihood ratio. (B) Forest plot of negative likelihood ratio. (C) Forest plot of diagnostic odds ratio.

and DOR were 0.14, 87.42, and 714.80, respectively. Our results reflected high diagnostic value of xTAG RVP FAST v2 for the detection of respiratory viral infections.

For subgroup analysis, we segregated the retrieved data into eight groups based on the type of virus [IFVA, PIV, hCoV, RSV, hMPV, AdV, IFVB and enteroviruses/rhinoviruses (EV/RV)]. Except for AdV and IFVB, the sensitivity of detecting other viruses was above 0.85. Radko, et al.[33] found the sensitivity of xTAG for IFVB to be the lowest at 0.75, compared with others over 0.9. However, more clinical data are required to show whether the xTAG RVP FAST v2 test is more sensitive to specific viruses. Meanwhile, we also employed bivariate boxplots to investigate outlier results. In one study, Esposito, et al.[28] found that xTAG RVP FAST v2 has low sensitivity to AdV: they had previously concluded that the Luminex NxTAG Respiratory Pathogen Panel was more suitable for detecting AdV.[34,35] Another outlier outcome was from a study in the detection of RV,[29] although this article included only 42 samples, which were too few for accurate analysis of sensitivity.

Research has shown that traditional detection methods are fraught with challenges and have several limitations: First, cultivation is time-consuming and laborious, and rapid detection of viral antigens is not highly sensitive to the majority of viruses. Second, although quantitative reverse transcription-PCR (RT-qPCR) has significantly improved the etiological diagnosis of respiratory infections at lower cost and less time required for analysis,[36] multiplex RT-qPCR is technically challenging and may lead to low sensitivity.[26] Therefore, multiple analyses involving amplification using suspension microarrays aiming to detect a series of respiratory viruses can provide practical solutions.[23,28] The xTAG RVP assay is based on suspension microarray technology, which enables the detection of a large number of targets in a single reaction.[26] Moreover, xTAG RVP FAST v2 can rapidly detect 19 respiratory viruses and their subtypes simultaneously in only 5 hours, providing benefits to manpower, detection efficiency, and determination of infectious pathogens in the shortest amount of time.[19,22]

For comparison, BioFire® (https://www.biofiredx.com/products/the-filmarray-panels/) has reported an overall sensitivity of 0.97 in their BioFire Respiratory 2.1 (RP2.1) Panel, targeting 22 respiratory viruses and bacteria. In respiratory viral analyses, RP2.1 showed a positive percent agreement of 97%.[37] The

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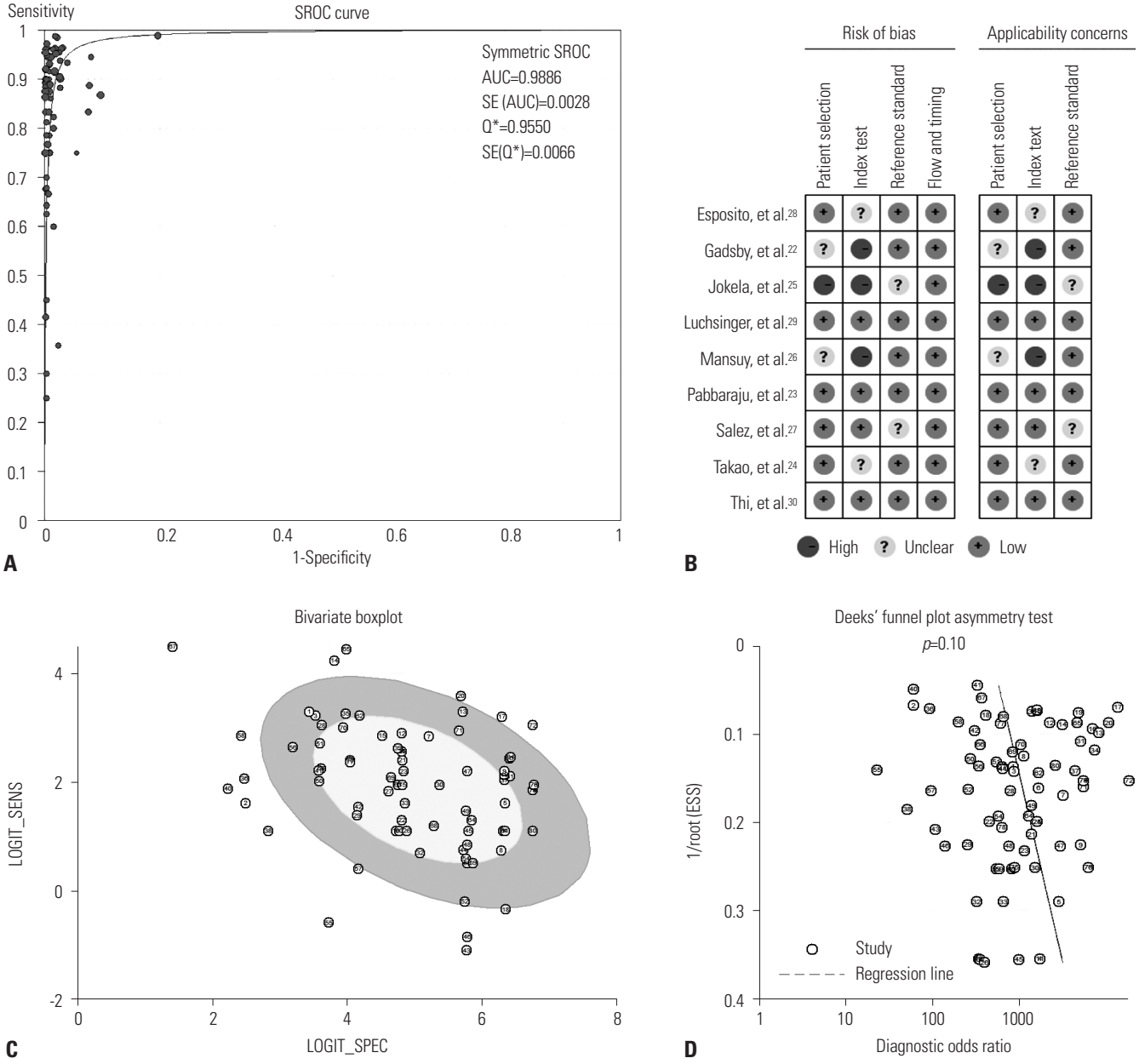

Fig. 3. Other analysis results. (A) SROC curve for xTAG RVP FAST v2 in detecting respiratory virus. (B) Quality evaluation summary of included studies. (C) Bivariate boxplot for outlier results. (D) Deek's funnel plot asymmetry test to assess publication bias. AUC, area under the curve; SROC, summary receiver operating characteristic; SENS, sensitivity; SPEC, specificity; ESS, effective sample size; SE, standard error.

relatively lower sensitivity of 0.88 obtained with xTAG RVP FAST v2 might be due to the extra manual operations required: to perform xTAG RVP FAST assay, samples collected from patients need extra off-board extraction and transference, which means longer hands-on time and a higher occurrence of nucleic acid damage. With BioFire RP2.1, samples can be directly added into a tube with minimal hands-on time and fewer processing manual steps, thereby reducing damage to organisms and ensuring higher sensitivity.[38]

Overall, there were several limitations in our study. We were unable to determine why different virus detection rates were different in xTAG RVP FAST v2, such as AdV and IFVB. Also, while two researchers evaluated article quality as a group, a third scholar was utilized in cases of disagreement; hence, we cannot ensure that there was no manual bias.

We assessed the efficacy of xTAG RVP FAST v2 for detecting respiratory viral infections. In summary, xTAG RVP FAST v2 appears to be a reliable and rapid diagnostic method for multiple respiratory viral infections. In clinical practice, xTAG RVP FAST v2 shows high diagnostic performance, especially for detecting IFVA, PIV, hCoV, hMPV, EV/RV, and RSV.

Table 2. Data Analysis for Individual Viruses

Virus	Reports*	Sensitivity (95% CI)	Specificity (95% CI)	PLR	NLR	DOR	AUC	*p* value†
IFVA	14	0.90 (0.87–0.93)	0.99 (0.99–1.00)	83.81	0.12	804.20	0.9739	0.455
PIV	12	0.91 (0.85–0.96)	0.99 (0.99–1.00)	97.83	0.14	906.07	0.9873	0.200
hCoV	11	0.87 (0.79–0.92)	1.00 (0.99–1.00)	129.46	0.22	1002.43	0.9966	0.419
RSV	10	0.93 (0.90–0.96)	0.99 (0.99–1.00)	97.19	0.10	1193.87	0.9907	0.982
hMPV	8	0.91 (0.83–0.96)	0.99 (0.99–1.00)	99.97	0.14	966.95	0.9941	0.240
AdV	7	0.84 (0.75–0.91)	0.98 (0.98–0.99)	43.13	0.14	405.39	0.9954	0.468
EV/RV	7	0.90 (0.87–0.92)	0.91 (0.90–0.92)	14.16	0.12	132.50	0.9737	0.224
IFVB	6	0.62 (0.51–0.72)	1.00 (1.00–1.00)	304.65	0.29	1249.49	0.9994	0.635

CI, confidence interval; PLR, positive likelihood ratio negative; NLR, likelihood ratio; DOR, diagnostic odds ratio; AUC, the area under the curve; IFVA, influenza virus A; PIV, parainfluenza virus; hCoV, human coronavirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; AdV, adenovirus; EV/RV, enteroviruses/ rhinoviruses; IFVB, influenza virus B.
*One study may have been included more than once due to different subtypes of a virus; †*p* value from Deek's funnel plot asymmetry test used to assess the publication bias.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

Conceptualization: Xu-Guang Guo. Data curation: Li-Min Xie. Formal analysis: Li-Min Xie and Xin Yin. Investigation: Li-Min Xie, Jian-Wen Su, Qin Huang, Jing-Hao Zhang, and Yin-Fei Huang. Methodology: Xu-Guang Guo. Project administration: Li-Min Xie. Resources: Xu-Guang Guo. Software: Li-Min Xie, Xin Yin, and Tian-Ao Xie. Supervision: Li-Min Xie. Validation: Li-Min Xie and Xin Yin. Visualization: Li-Min Xie and Xin Yin. Writing—original draft: all authors. Writing—review & editing: Li-Min Xie and Xin Yin. Approval of final manuscript: all authors.

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