DIAGNOSTIC ACCURACY OF SALIVA REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) COMPARED TO NASOPHARYNGEAL SWAB REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) IN THE DETECTION OF SARS-COV-2 IN PEDIATRIC PATIENTS AGES 0-18 YEARS OLD : A META-ANALYSIS

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ABSTRACT

OBJECTIVES: To determine the diagnostic accuracy of Saliva RT-PCR in the detection of SARS-COV-2 in pediatric patients ages 0-18 years old, compared to the nasopharyngeal RT-PCR swab.

METHODOLOGY: A metanalysis was done to synthesize the diagnostic accuracy of saliva RT-PCR compared to the nasopharyngeal RT-PCR in the detection of SARS-COV 2 in pediatric patients ages 0-18 years old. Five studies published from January to September 2021 were analyzed using the "midas" command of STATA14. MIDAS command is a comprehensive program of statistical and graphical routines for undertaking meta-analysis of diagnostic test performance in Stata. The index and reference tests (gold standard) are dichotomous. Primary data synthesis is performed within the bivariate mixed-effects regression framework focused on making inferences about average sensitivity and specificity.

RESULTS: The World Health Organization's acceptable sensitivity and specificity for products used in COVID-19 diagnostics is ≥ 80% and ≥ 97% respectively. The results of this metanalysis showed the pooled sensitivity of Saliva RT-PCR as compared to the Nasopharyngeal RT-PCR is at 87% (81-92% at 95% CI) and the pooled specificity is at 97% (95% CI: 96-98%).

CONCLUSIONS: This metanalysis demonstrates that saliva can be used as an alternative specimen for SARS-COV-2 diagnostic testing in children. Aside from the acceptable pooled specificity and sensitivity, the use of saliva offers several advantages. However, the authors recommend to include more studies for future metanalysis research, to further increase sample

size, and to include both symptomatic and asymptomatic pediatric age group participants. A future prospective research study comparing the two diagnostic modalities is likewise recommended

Keywords: *COVID-19, SARS-COV-2, Nasopharyngeal RT-PCR, Saliva RT-PCR, Children, 0-18 years old*

INTRODUCTION

A day before the start of 2020, an atypical respiratory disease similar to pneumonia and/or influenza was reported to the World Health Organization (WHO) Country Office in China. It was first detected in clusters in Wuhan City, Hubei Province, China. Later, it was discovered that this disease is caused by the Severe Acute Respiratory Syndrome Coronary Virus 2 (SARS COV-2)[1]. Within a month, the new virus was discovered to be highly-contagious and rapidly spread into many countries, with approximately 6000+ confirmed cases by January 2020. In January 30, 2020, the WHO declared the outbreak to be a Public Health Emergency of International Concern and by March 11, it was escalated as a pandemic upon the declaration of the WHO Director General. The WHO[2] officially named the new coronavirus disease as COVID-19 by February 11, 2020 and, not long after, confirmed cases spread across the globe, causing countries to enforce lockdowns to curb infection and deaths.

Testing has become a main defense tactic against the COVID-19 virus, with the reverse-transcriptase polymerase chain reaction (RT-PCR)[3] test through nasopharyngeal swab specimen as the main method currently utilized. An accurate diagnosis is important in the management and prevention of transmission of COVID-19 both in the adult and pediatric population. Like other parts of the world, the Philippines has been facing challenges in fighting COVID-19. Apart from the lack of easy and universal access to treatments and vaccines, Nasopharyngeal RT-PCR, which is the current gold standard in the diagnosis of COVID-19, has some several drawbacks starting from sample collection that usually causes pain and discomfort especially in the children and elderly up to the increased risk for viral transmission to the healthcare

worker brought about by reflex sneezing or coughing.

Nasopharyngeal sampling requires significant human resources, time, and preparation, resulting in testing bottlenecks and the risk of transmission in overcrowded testing sites. Furthermore, the unpleasantness of the procedure and the long wait times for swab collection and results may deter some people from getting tested or from repeating negative tests. Thus, innovative testing techniques that utilize the tried and tested RT-PCR method are urgently needed to quickly classify cases, reduce waiting times, and promote mass screening.

A novel testing technique that can be a viable alternative to nasopharyngeal swab is saliva sampling. The pathophysiology behind the use of saliva for testing lies in the high salivary gland expression of host angiotensin-converting enzyme, which regulates the host receptor-cellular entry of SARS-CoV-2[25]. In addition, It has the advantage of being simple and painless to obtain, requiring no qualified personnel and even possibly allowing self-sampling. However, comparisons between real-time PCR results from salivary and nasopharyngeal samples show variations, with most finding greater sensitivity and lower RT-PCR counts in nasopharyngeal swab samples[4-6], while others find greater sensitivity in saliva samples[7-8].

A study done by El-Sharkawy, et.al published last March 2022 compared the performance of saliva and upper respiratory swab in the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Paired saliva and anterior nares specimens were collected from a largely asymptomatic cohort of students, faculty, and staff from the University of Pennsylvania. Paired saliva and combined nasopharyngeal/oropharyngeal (NP/OP) specimens were also collected from hospitalized patients with symptomatic COVID-19 following confirmatory testing. All study samples were tested by real-time PCR in the Hospital of the University of Pennsylvania. In the university cohort, positivity rates were 37 of 2500 for saliva (sensitivity, 86.1%) and 36 of 2500 for anterior nares (sensitivity, 83.7%), with an overall agreement of 99.6%. In the hospital study cohort, positivity rates were 35 of 49 for saliva (sensitivity, 89.3%) and 28 of 49 for NP/OP (sensitivity, 75.8%), with an

overall agreement of 75.6%. A larger proportion of saliva than NP/OP samples tested positive after 4 days of symptom onset in hospitalized patients. This showed that saliva has an acceptable sensitivity and is comparable to upper respiratory swab, supporting the use of saliva for SARS-CoV-2 detection in both symptomatic and asymptomatic populations.

However, a study by Mestdagh, Et. Al[27] published last July 2021 also compared saliva specimens and nasopharyngeal (NP) swabs with respect to sensitivity in detecting SARS-CoV-2. In this study, a nasopharyngeal and two saliva specimens (collected by spitting or oral swabbing) were obtained from >2500 individuals. All samples were tested by RT-qPCR, detecting RNA of SARS-CoV-2. The test sensitivity was compared on the two saliva collections with the nasopharyngeal specimen for all subjects and stratified by symptom status and viral load, of the 2850 patients for whom all three samples were available, 105 were positive on NP swab, whereas 32 and 23 were also positive on saliva spitting and saliva swabbing samples, respectively. The sensitivity of the RT-qPCR to detect SARS-CoV-2 among NP-positive patients was 30.5% (95% CI, 1.9%e40.2%) for saliva spitting and 21.9% (95% CI, 14.4%e31.0%) for saliva swabbing. However, when focusing on subjects with medium to high viral load, sensitivity on saliva increased substantially: 93.9% (95% CI, 79.8%e99.3%) and 76.9% (95% CI, 56.4%e91.0%) for spitting and swabbing, respectively, regardless of symptomatic status. This result suggests that saliva cannot readily replace nasopharyngeal sampling for SARS-CoV-2 diagnostics but may enable identification of the most contagious cases with medium to high viral loads.

Given the conflicting findings in both in the adult and pediatric population, a meta-analysis is warranted to find consensus on the diagnostic accuracy of saliva sample versus nasopharyngeal swab.

This study summarizes existing literatures which compared the diagnostic accuracy of saliva as compared to the nasopharyngeal swab RT-PCR in detecting SARS-CoV-2 in the pediatric population ages 0-18 years old. A favorable result from this study will provide additional information to the current guidelines used in the diagnosis of COVID-19 in the pediatric population

which in turn can bring us a step closer in ending this Pandemic.

This study aims to determine the diagnostic accuracy of Saliva RT-PCR in the detection of SARS-COV2 in pediatric patients ages 0-18 years old as compared to the Nasopharyngeal RT-PCR swab.

MATERIALS AND METHODS

Research Design

A meta-analysis was done to compare the diagnostic accuracy of saliva RT-PCR and nasopharyngeal RT-PCR in the detection of SARS-COV 2 in pediatric patients ages 0-18 years old.

Search Strategy and Study Identification

Pubmed, Medline, Google Scholar (first 1000 articles), and ResearchGate were searched using keywords (saliva) AND (nasopharyngeal OR nasopharynx) AND (RT-PCR OR "Reverse transcription polymerase chain reaction") AND (COVID-19 OR SARS-COV-2) AND (Children 0-18 years old OR Pediatric population). Forward search of literatures citing the included studies were done for possible additional studies. Backward review of other references cited in included studies were also done. Searches covered all studies published until September 15, 2021.

Eligibility Criteria

I. Types of studies

Diagnostic accuracy studies which described the sensitivity and specificity of saliva RT-PCR when compared to nasopharyngeal swab as gold standard in detecting SARS-CoV-2 were included. All studies until September 15, 2021and available in the English language were included. Excluded were studies which included both adult and children.

II. Types of participants

Only studies which involved individuals ages 0-18 years old diagnosed or suspected to have COVID-19, and those screened before surgery and other procedures, were considered eligible for this analysis.

DATA COLLECTION AND DATA ANALYSIS

Selection of studies and quality assessment

Two review authors screened the titles and abstracts of articles identified by the search strategy as relevant using the inclusion criteria. Studies deemed applicable for

possible inclusion were then evaluated using full article copies in terms of objectives, methodology, reporting of outcomes and appropriateness for final inclusion.

Study quality was assessed using QUADAS-2 tool (quality assessment for diagnostic accuracy study) of the Review Manager version 5.4 software. Using this tool, each study was assessed in terms of representativeness of samples, selection criteria, reference standard, and flow/timing of outcome confirmation.

Data extraction and management

Data from studies were extracted into Microsoft Excel and STATA14. Information included were author, year of publication, setting, total sample size, number of patients included, sensitivity, specificity, and outcomes reported (true positives, true negatives, false positives, and false negatives). Two reviewers performed the data extraction and disputes were broken by a third reviewer's decision.

Statistical Analysis and data synthesis

The studies were analyzed using the "midas" command of STATA14. Midas is a comprehensive program of statistical and graphical routines for undertaking meta-analysis of diagnostic test performance in Stata. The index and reference tests were dichotomous. Primary data synthesis was performed within the bivariate mixed-effects regression framework focused on making inferences about average sensitivity and specificity. The pooled ROC for all studies were presented. The following guidelines was used for the interpretation of intermediate area under ROC values: low (0.5>= AUC <= 0.7), moderate (0.7 >= AUC <= 0.9), or high (0.9 >= AUC <= 1) accuracy.

RESULTS

In the primary search through databases, a total of 986 abstracts was screened, while 949 were excluded. Out of the 37 full-text articles reviewed, only five studies satisfied the inclusion and exclusion criteria of the study.

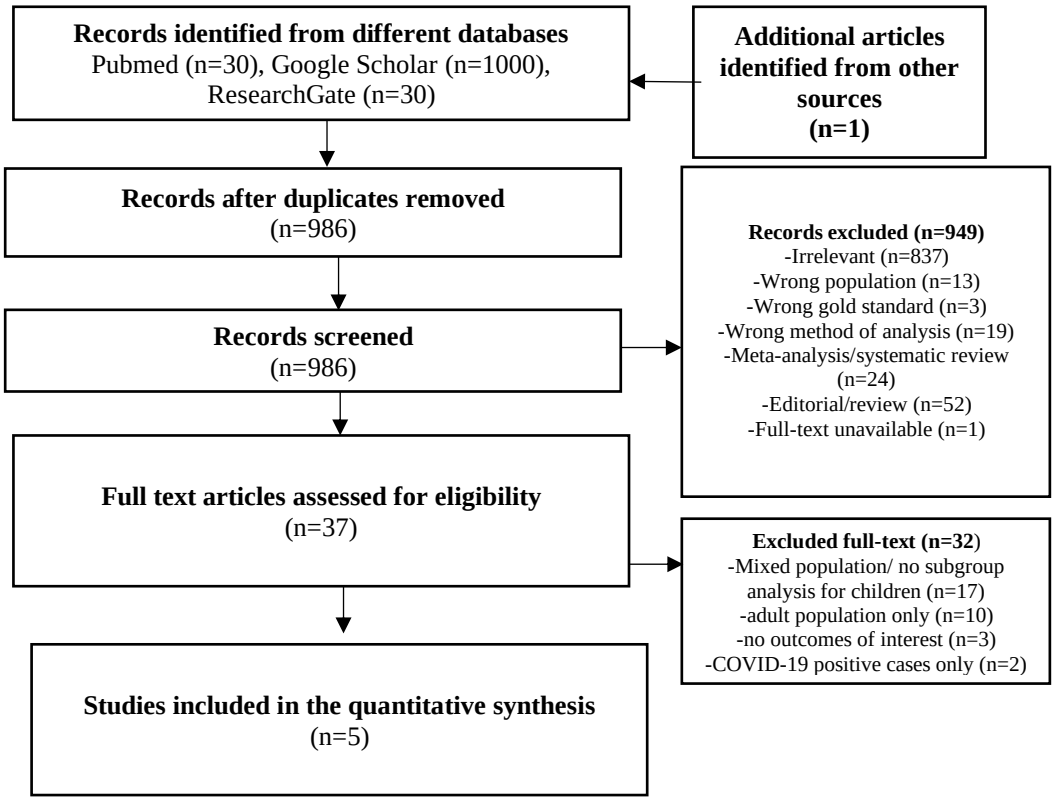

Figure 1. PRISMA Flow Chart of Literature Search

Study Characteristics

The characteristics of the studies included are presented in Table 1. All studies were published in 2021 coming from five different countries. The total number of patients is 937 with a total sample of 946. The mean age ranges from 3.8 to 13 years old. Males comprised 46-58.2% of the population. All saliva specimens were collected on the same day of the nasopharyngeal swab except for one study (Alenquer, 2021) as shown in Table 2.

Table 1. Characteristics Of Included Studies

Author, Year	Country	Population	No. of patients	No. of samples	Mean age (Range)	% male
Al Suwaidi, 2021	UAE	Presenting for COVID-19 screening: confirmed COVID-19 patients, presence of presumptive symptoms or testing for return to school.	476	485	10.8 (3-18)	58.2%
Alenquer, 2021	Portugal	Admitted to hospital for COVID-19 symptoms or causes non-related to COVID-19	85	85	3.8 (<10)	54.1%
Felix, 2021	Brazil	Suspected COVID-19 (mild symptoms)	50	50	10.24 (range not specified)	46%
Huber, 2021	Switzerland	Patients with COVID-19 symptoms and asymptomatic patients with relevant exposure to COVID-19 Excluded hospitalized patients	170	170	Median: 13 (5-17)	51.8%
Laura, 2021	Mexico	Hospitalized patients who showed respiratory symptoms while recovering from a disease other than COVID-19, and non-probable COVID-19 patients who attended to the hospital for routine clinical analyses before a programmed surgery	156	156	Median: 11 (5-18)	50%

Table 2. Specimen Collection Details

	Saliva specimen	Timing of assessment: Saliva and NP swab specimen
Al Suwaidi, 2021	-Abstinence from food or drink for at least 30 minutes -1-3 ml saliva, self-collected -Participants were asked to close their mouths, allow saliva to pool in the mouth for 1-2 minutes, and gently spit into the provided sterile container	Same day
Alenquer, 2021	-Abstinence from food or drink for at least 30 minutes -At least 1ml saliva collected with help of a healthcare worker -Participants were asked to pool saliva in the mouth and gently spit it into a sterile container without coughing or clearing their throats. For children under the age of 1 year, saliva was gently aspirated from the mouth with a suction tube.	Saliva samples collected within 24 or 48 hours from NP swab collection
Felix, 2021	-1 ml of saliva spit into a sterile container	Same day
Huber, 2021	-Abstinence from food or drink not performed -0.5-1 ml saliva -"Basic": clear throat thoroughly and collect saliva one or two times into the same tube -"Enhanced": clear throat three times and collect saliva into the same tube	Same day
Laura, 2021	-spit 5 times into a sterile container -not instructed to cough out or try to enrich samples with sputum	Same day

Table 3 shows the results of the various studies in terms of Specificity, Sensitivity, True Positive, False Positive, False Negative and True negatives. As prevalence increases, Positive Predictive Value (PPV) increases and Negative Predictive Value (NPV) decreases. In this metanalysis, we can see that the study done by (Al-Suwaidi,2021) has the lowest PPV since it is the only study that included asymptomatic individuals unlike the other remaining studies which mostly tested for symptomatic patients or close contact of COVID-19 patients.

Table 3. Results of Included Studies

Author, Year	Sensitivity	Specificity	TP	FP	FN	TN
Al Suwaidi, 2021	87.7% (95% CI: 78.5-93.9)	98.5% (95% CI: 96.8-99.5)	71	6	10	398
Alenquer, 2021	84.8% (95% CI: 71.8-92.4)	100% (95% CI: 91-100)	39	0	7	39
Felix, 2021	75% (95% CI: 35-97)	95.2% (95% CI: 84-99)	6	2	2	40
Huber, 2021	93.3% (95% CI: 78-99)	96.4% (95% CI: 92-99)	28	5	2	135
Laura, 2021	82.3% (95% CI: 56.6-96.2)	95.6% (90.8-98.4)	14	6	3	133

Figure 2 shows Pooled sensitivity of 87% (95% CI: 81-91%) while Pooled specificity of 97% (95% CI: 96-98%).The WHO's acceptable sensitivity and specificity for products used in COVID-19diagnostics is ≥ 80% and ≥ 97% respectively. On the other hand, minimal heterogeneity (I2=0%) was observed for sensitivity, and moderate heterogeneity (I2=35%) for specificity. Both are not considered significant and does not affect the overall study

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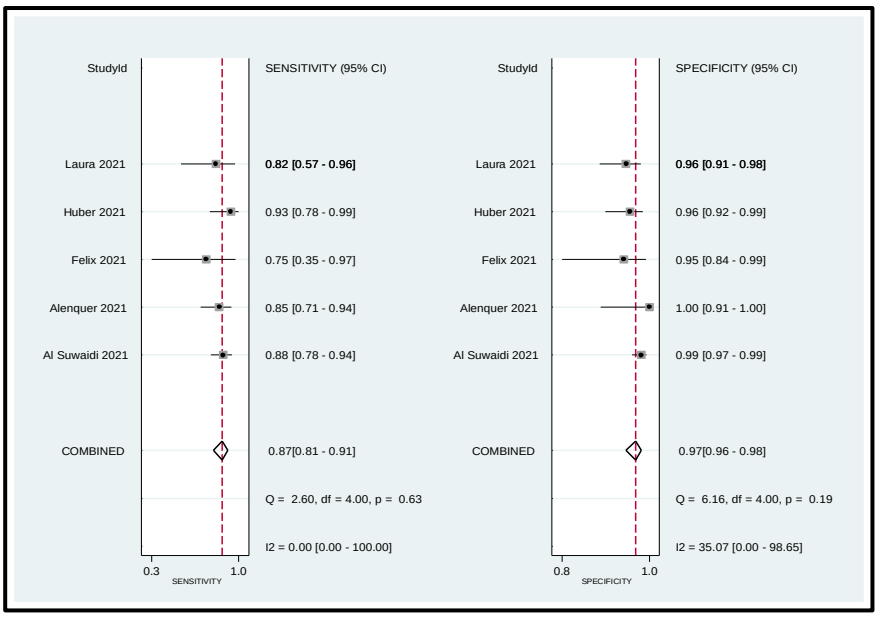

Figure 2 Forest plot showing the sensitivity and specificity of saliva RT-PCR in detecting COVID-19

Figure 3 shows that pooled AUC has high accuracy of 0.91 (95% CI: 0.88-93) while Figure 4 reveals that the pooled AUC despite the Alenquer study being excluded in the analysis. AUC remained high at 0.91 (0.88-0.93)

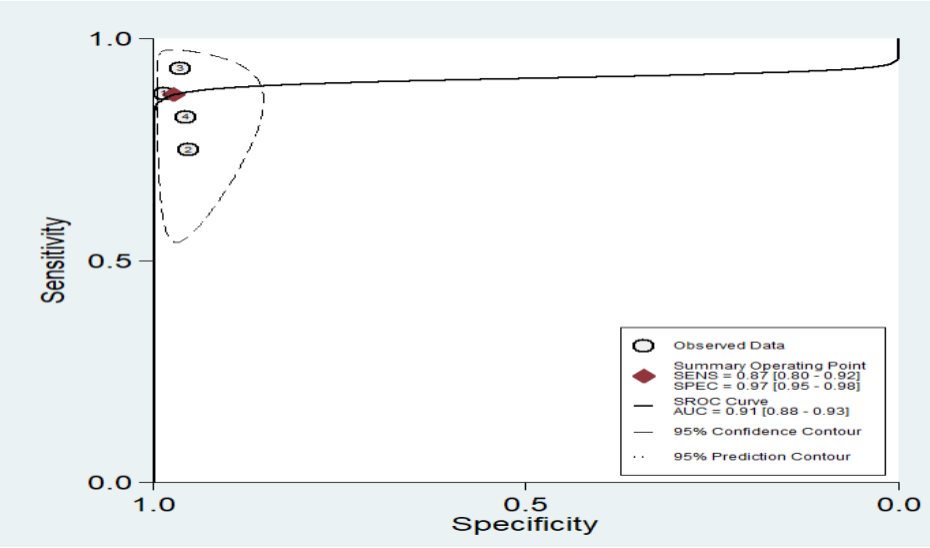

Figure 3. Pooled Area under the curve of 0.91 (95% CI: 0.88-093)

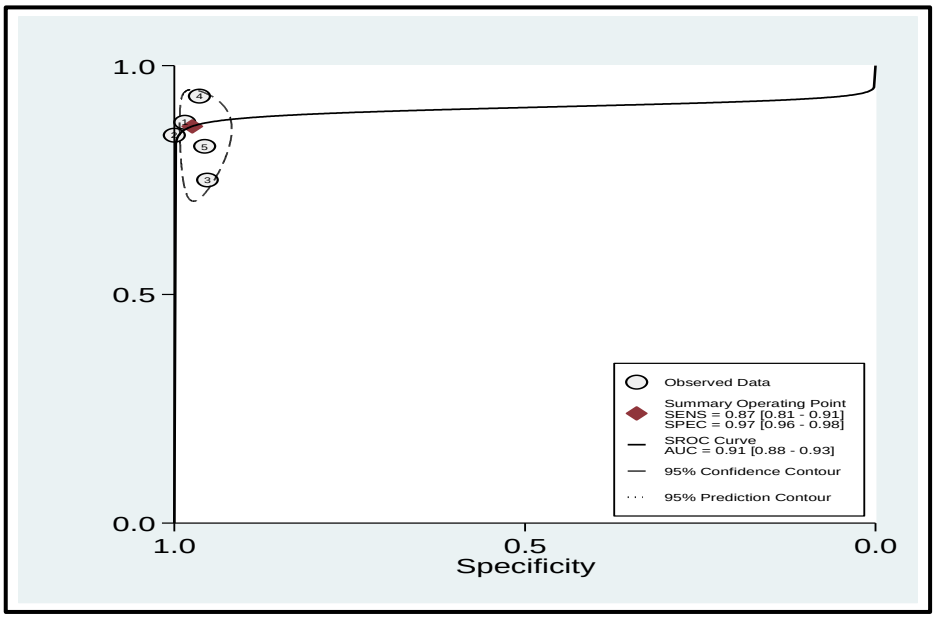

Figure 4. Pooled Area under the curve after the Alenquer study was excluded in the analysis.

Risk of bias

Figure 5 and 6 shows the risk bias and applicability of the study. Regarding patient selection, only two studies had low risk of bias, and only one study had low concern in terms of applicability. Risk of bias for the index test was low for three studies, and applicability concerns were low for four studies. Risk of bias for the reference standard was unclear from one study. However, all studies showed low concern for applicability. Only one study had high risk of bias for flow and timing.

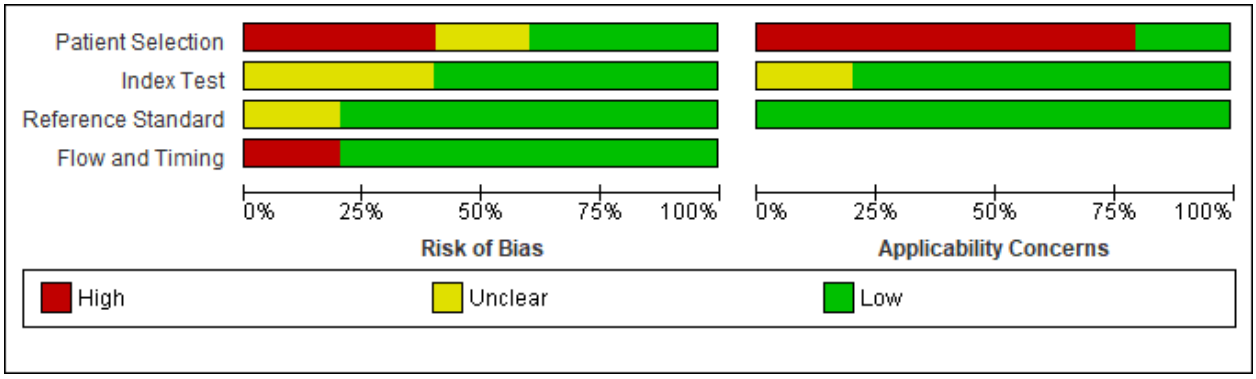

Figure 5 Risk of bias and applicability concerns graph: review authors' judgments about each domain presented as percentages across included studies

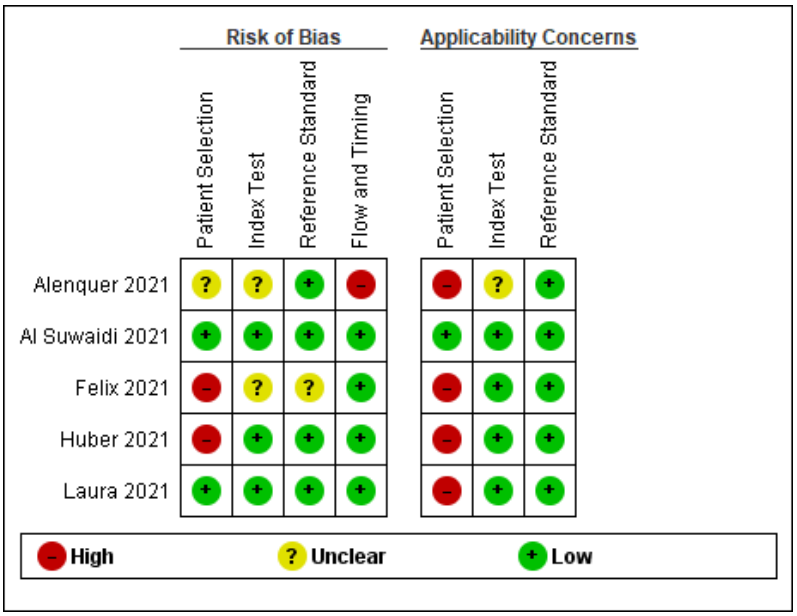

Figure 6 Risk of bias and applicability concerns summary: review authors' judgments about each domain for each included study

The Funnel plot as shown in Figure 7, revealed an asymmetric test results which signifies a Publication bias. However, it must be noted that this metanalysis only included less than 10 studies hence the power of the test may be too low to distinguish chance from true asymmetry.

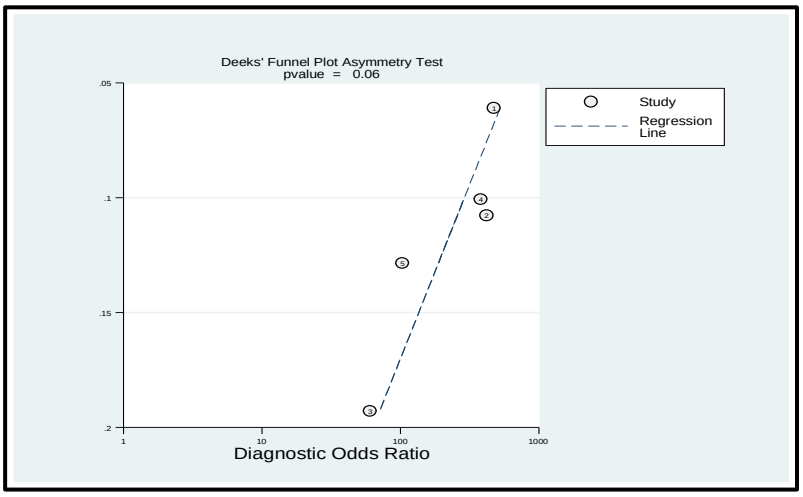

Figure 7. Deek's funnel plot asymmetry test

DISCUSSION

Accuracy of Saliva RT-PCR compared to Nasopharyngeal RT-PCR

The WHO's acceptable sensitivity for products used in COVID-19diagnostics is ≥ 80%. In this metanalysis, It was noted that pooled sensitivity of Saliva RT-PCR as compared to the Nasopharyngeal RT-PCR is at 87% (81-92% at 95% CI) which is within the acceptable range.

In terms of specificity, the standard set by the WHO is at ≥ 97%. In this study, it can be seen that the pooled specificity of saliva RT-PCR is at 97% which falls within the acceptable specificity set by the WHO.

Strengths and weaknesses

Heterogeneity of studies

Heterogeneity is defined as the variation in study outcomes between studies which is usually caused by differences in population characteristics, methodology, and other factors. It is determined by analyzing the sensitivity and specificity results. In this metanalysis, the pooled sensitivity showed minimal heterogeneity (p = 0.19). This can also be seen in the Forrest plot (Figure 2)

which shows that sensitivity points are not distant to each other showing low variation and consistency within the results.

On the other hand, there is noted heterogeneity (12=359%) on the specificity analysis. As seen in the Forrest plot (Figure 2), the specificities of each study are inconsistent as shown by the distance of specificity values of each study. Significance of heterogeneity can be tested by measuring its p-value. A p-value of >0. 1 (0.19 on this metanalysis) is considered not significant. Therefore, it can be stated that heterogeneity, though present, is not significant and will not affect the overall study.

The predictive value quantifies the probability that a positive test result correctly identifies the presence of infection and a negative test result correctly identifies the absence of infection. This requires knowledge of not only the sensitivity and specificity of the test but the prevalence of the condition. The effect of prevalence on predictive values is considerable. As prevalence increases, Positive Predictive Value (PPV) increases and Negative Predictive Value (NPV) decreases. In this metanalysis, we can see that the study done by (Al-Suwaidi,2021) has the lowest PPV since it is the only study that included asymptomatic individuals unlike the other remaining studies which mostly tested for symptomatic patients.

Risk of bias

Some risks of bias are identified among the selected studies. In terms of patient selection, most of the studies focused on testing symptomatic parents. This may pose as risk for bias since symptomatic patients have higher probability of testing positive for COVID-19.This may also affect the applicability in testing asymptomatic patients. Only one study (Al-Suwaidi,2021) tested asymptomatic patients. Hence it is recommended to perform diagnostic studies which will cater to both symptomatic and asymptomatic patients.

A publication bias was also observed based on the Deek's funnel asymmetry test, however, since there were only less than 10 studies included in this metanalysis, the power of the test may be too low to distinguish chance from true asymmetry.

CONCLUSION AND RECOMMENDATIONS

Despite the strengths and weaknesses presented, the data gathered from the metanalysis demonstrate that saliva specimen can be used as an alternative for SARS-COV-2 diagnostic testing in children as demonstrated by the pooled specificity and sensitivity. However, the acceptable positive and negative predictive of the studies included in the metanalysis may not be reflective of the general pediatric population since most patients tested were symptomatic or close contacts of COVID-19 patients.

There are limitations identified considering the number of studies included. The authors recommend to include more studies for future metanalysis research, to further increase sample size, and to include both symptomatic and asymptomatic pediatric age group participants. A future prospective research study comparing the two diagnostic modalities is likewise recommended.

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