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A Novel Medical Technology for Rapid Molecular Diagnostics (Nicking Enzyme Amplification Reaction [NEAR]) – A Systematic Literature Review, Meta-Analysis

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Introduction and Objectives

- The RT-PCR method is often considered as the "gold standard" for detecting SARS-COV -2 (COVID-19). However, a limitation of PCR is the "time to result."
- With the rapid molecular diagnostic test based on the nicking enzyme amplification reaction (**NEAR technology**), results can be obtained in a very short time (3-15 minutes). Thanks to the technology used to obtain an accurate result, clinicians can make evidence-based decisions in a short time, i.e., during the patient visit. NEAR test is indicated in **symptomatic patients suspected of COVID-19 infection, in whom symptoms appeared within 7 days**.
- NEAR test is also designed for detection of influenza A and B, RSV, and group A streptococci.
- The objective of this systematic literature review (SLR) was to evaluate the diagnostic efficacy of one of the rapid molecular diagnostic tests based on NEAR technology (intervention) for the qualitative detection of SARS-CoV-2 (COVID -19) infection compared to RT-PCR.

Methods

- We systematically searched EMBASE, Medline, and the Cochrane Library through November 31, 2021, for all prospective studies of the diagnostic efficacy of the assessed intervention compared with RT-PCR.
- Only studies, where the intervention was used according to the **valid protocol recommended by the manufacturer** (Abbott Laboratories) were considered for inclusion. Predefined eligibility criteria are listed in Table 1.

Table 1. Study eligibility criteria

PICOS	Eligibility criteria
Population	Acute infectious disease of the respiratory system caused by infection with SARS-CoV-2 (COVID-19) virus - symptomatic patients
Intervention	NEAR diagnostic test - isothermal molecular technology
Comparator	Real-time polymerase chain reaction (RT-PCR) – „Gold standard"
Outcomes	At least one of the following clinically relevant endpoints: sensitivity; specificity
Study design	Prospective studies on human samples
Study protocol (diagnostic method)	• Dry swab collected by investigator – undiluted in universal transport media (UTM) • Samples for intervention and control taken from the same place, i.e. from the nose, nasopharynx, etc. • Fresh" samples - tested shortly after collection, without freezing
Restriction	• English language

Abbreviations: NEAR*: Nicking Enzyme Amplification Reaction;* RT-PCR*: Real-time polymerase chain reaction;* UTM*: Universal transport media*

- Abstracts and full-text articles were reviewed by two independent reviewers. Data were extracted by one reviewer and validated by another. The key extraction fields included the following: study design and characteristics, baseline patients' characteristics, control („gold standard"), place of swab collection, the time from taking the swab to taking the test, time from the onset of symptoms till testing, and number of samples.
- The studies included in the meta-analysis examined diagnostic parameters such as: true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN). The calculations were based on a table with four fields containing the number of patients with positive (+) and negative (-) test results assessed in patients with the disease (+) or without disease state (-) diagnosed with a reference test.

Table 2. Diagnostic Testing Accuracy

Reference test/Assessed test	Disease present	Disease not present
Positive result of assessed test	True positive (A)	False positive (B)
Negative result of assessed test	False negative (C)	True negative (D)

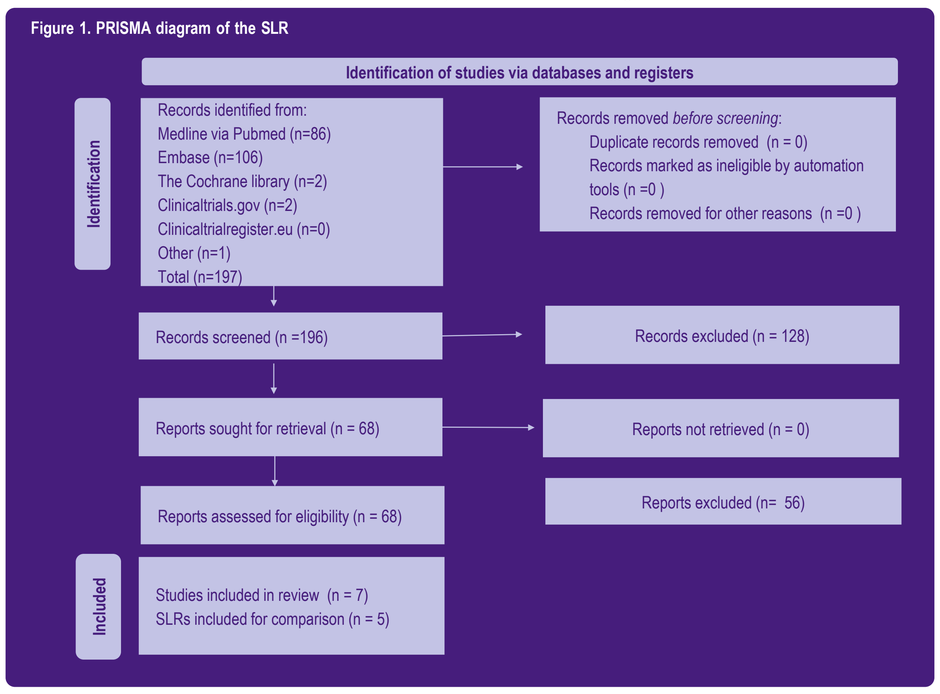
- Diagnostic efficacy parameters, including sensitivity and specificity values for all interventions assessed, were calculated only when complete diagnostic data were available (four-field table). Sensitivity is the proportion of true positives tests out of all patients with a condition [2]: Sensitivity=(True Positives (A))/(True Positives (A)+False Negatives (C)). Specificity is the percentage of true negatives out of all subjects who do not have a disease or condition [2]: Specificity=(True Negatives (D))/(True Negatives (D)+False Positives (B)).
- After completing four-field tables for all included 7 studies meta-analysis was performed of sensitivity, specificity and percentage of false positives for 3 scenarios:
  1. **Base case:** All included studies
  2. **Sensitivity analysis 1**: Meta-analysis considered only those studies, which provided information on the time of symptoms till testing
  3. **Sensitivity analysis 2**: Meta-analysis included only those studies, which considered RT-PCR methods used in clinical practice in Poland.
- All calculations were performed using the online application MetaDTA: Diagnostic Test Accuracy Meta-Analysis v2.01 (August 17, 2021) [3].

Results

Included studies

- Of 196 records, 7 studies met the inclusion criteria and 5 SLRs were eligible for comparison. The most common reason for exclusion at the full-text stage was a lack of adherence to the study protocol recommended by the manufacturer (Figure 1).

Figure 1. PRISMA diagram of the SLR



- Of the 7 studies, 4 were conducted in the hospital (including 2 in the emergency department, 1 included community and hospital patients, 1 was conducted in COVID-19 quarantine facilities, and 1 included outpatients [Table 2].
- Nasopharyngeal (NP) samples were used in 5 studies. In 2 studies, samples were collected from the pharynx (T) and nose (N), respectively. The time from collection of the swab to performance of the test was reported in 2 studies and was less than 1 hour. The time from onset of symptoms to testing was reported in 3 studies and was less than 7 days.

Table 2. Summary of included studies (n=7)

Study	Control	Setting	Place of swab collection	The time from taking the swab to taking the test	Time from the onset of symptoms till testing	N of samples
Urgent Care Clinic study 2020 [4]	Roche Cobas® SARS-CoV-2	Urgent care clinics	NP	NR	Less than 7 days	256
Graham 2021 [5]	Xpert Xpress SARS-CoV-2	Academic hospitals	N	NR	Less than 7 days	1043
Mahmoud 2021 [6]	Roche Cobas® SARS-CoV-2	COVID-19 quarantine facilities	NP	NR	NR	686
Meletis 2021 [7]	Abbott RealTime SARS-CoV-2	Emergency department	NP	NR	NR	30
NguyenVan 2021 [8]	Simplexa COVID-19	Emergency department	NP	NR	NR	395
*Stokes 2021** [9]	Roche Cobas® SARS-CoV-2	Community and hospital	T	(<1h)	Less than 7 days	62
Tu 2021 [10]	Hologic Panther Fusion® SARS-CoV-2	Ambulatory	NP	<1h (approx. 15 min.)	NR	965

* results for the subpopulation of patients in whom symptoms occurred within 7 days and the sample was analyzed within 1 hour of swab collection (this is the population according to the test protocol provided by the manufacturer)

Abbreviations: N*: Nose;* NP*: Nasopharyngeal ;* NR*: Not reported;* RT–PCR*: Real-time polymerase chain reaction;* T*: Throat*

Meta-analysis: The base case (Prospective studies on symptomatic patients): The search conducted allowed the identification of clinical studies evaluating the diagnostic efficacy of the NEAR test for the qualitative detection of acute respiratory infectious disease caused by COVID -19 viral infection compared to the RT-PCR method.

- In 7 prospective studies, the sensitivity and specificity of the NEAR diagnostic test for the qualitative detection of acute respiratory infectious disease caused by SARS-CoV-2 infection with symptoms were evaluated in comparison to the "gold standard": the RT-PCR method. Three studies reported the time between the onset of disease symptoms and the collection of the swab: *Urgent Care Clinic study 2020*: average 4.1 days*; Graham 2021*: median: 1 day (range 0 - 60 days); *Stokes 2021*: median: 6.9 days (1-17 days). The remaining studies did not report the time between the onset of disease symptoms and the collection of the smear, which can be considered a limitation of the studies.
- Detailed data on the assessed endpoints in individual studies are presented in the table below [Table 3].

Table 3. Diagnostic effectiveness of the NEAR test in relation to the RT-PCR method in prospective studies on symptomatic patients with suspected acute infectious respiratory disease caused by SARS-CoV-2 (COVID-19) infection (N=7)

Study, year	TP	FN	FP	TN	N	Sensitivity	Specificity
Urgent Care Clinic study 2020	29	0	1	226	256	1.000	0.996
Graham 2021	0	0	1	1042	1043	NaN	0.999
Mahmoud 2021	158	8	16	504	686	0.952	0.969
Meletis 2021	12	2	0	18	32	0.857	1.000
NguyenVan 2021	151	3	6	235	395	0.981	0.975
*Stokes 2021**	50	1	4	7	62	0.980	0.636
Tu 2021	21	2	0	942	965	0.913	1.000

Results for the subpopulation of patients in whom symptoms occurred within 7 days and the sample was analyzed within 1 hour of swab collection (this is the population according to the test protocol provided by the manufacturer)

Abbreviations: FN*: False negative;* FP*: False positive;* N*: Number of samples; NaN: uncountable value;* TN*: True negative;* TP*: True positive*

- Using the data from the four-field table reported in all 7 clinical trials for the NEAR test compared with the RT-PCR method, a meta-analysis of the sensitivity and specificity of the tests was performed. The results of the meta-analysis of 7 clinical studies are presented in Table 4 below.

Table 4. Results of the meta-analysis of the sensitivity and specificity of the NEAR test in relation to the RT-PCR method assessed in prospective studies among patients with suspected acute respiratory infectious disease caused by SARS-CoV-2 (COVID-19) infection: Base case (N=7); Sensitivity analysis 1: Studies of patients with symptoms appearing within 7 days (N=3); Sensitivity analysis 2: Studies in which the standard is one from RT-PCR methods used in Poland (N=4)

Parameter	Base case			Sensitivity analysis 1			Sensitivity analysis 2		
	The result of the meta-analysis	2.5% CI	97.5% CI	The result of the meta-analysis	2.5% CI	97.5% CI	The result of the meta-analysis	2.5% CI	97.5% CI
Sensitivity	0.956	0.918	0.976	0.987	0.917	0.998	0.971	0.941	0.986
Specificity	0.995	0.946	0.999	0.989	0.989	0.989	0.966	0.849	0.993
Percentage of false positives	0.005	0.001	0.054	0.011	0.011	0.011	0.034	0.007	0.151

Base case: The sensitivity of the NEAR test for the diagnosis of acute infectious respiratory disease caused by COVID -19 infection in symptomatic patients compared with the RT-PCR method was **0.956** (95% CI: 0.918 - 0.976) and the specificity was **0.995** (95% CI: 0.946 - 0.999).

Sensitivity analysis 1 (closest to compliance with the manufacturer's protocol): The meta-analysis included 3 prospective studies in symptomatic patients not more than 7 days after the onset of symptoms: *Urgent Care Clinic study 2020*; *Graham 2021*; *Stokes 2021*. In the identified studies, the "gold standard" was the RT-PCR method performed on the following models: Roche Cobas® SARS-CoV-2 or Cepheid Xpert Xpress SARS-CoV-2.

- The sensitivity of the NEAR test for the diagnosis of acute infectious respiratory disease caused by SARS-CoV-2 infection in symptomatic patients compared with the RT-PCR method was 0.987 (95% CI: 0.917 - 0.998) and the specificity was 0.989 (95% CI: 0.989 - 0.989)

Sensitivity analysis 2: Meta-analysis included only prospective studies with symptomatic patients, which considered RT-PCR methods used in clinical practice in Poland.

- The sensitivity analysis included 4 prospective studies on symptomatic patients, in which one of the RT-PCR methods used in Poland was the standard: *Urgent Care Clinic study 2020; Stokes 2021*; *Mahmoud 2021*; *NguyenVan 2021.*
- Time from symptom onset in sampled patients was not reported.
- In the identified studies, the "gold standard" was the RT-PCR method performed on the following models: Roche Cobas® SARS-CoV-2, Cepheid Xpert Xpress SARS-CoV-2 or Simplexa COVID-19.
- The sensitivity of the NEAR test for the diagnosis of acute infectious respiratory disease caused by SARS-CoV-2 infection in symptomatic patients compared with the RT-PCR method was 0.971 (95% CI: 0.941 - 0.986) and the specificity was 0.966 (95% CI: 0.849 - 0.993)

Comparison with other identified SLRs: As a result of searching medical information databases, 5 systematic reviews with meta-analysis were identified that assessed the diagnostic effectiveness of NEAR test for the qualitative detection of acute respiratory infectious disease caused by a virus infection SARS-CoV-2 (COVID-19): *Subsoontorn 2020* [11]; *Van Walle 2020* [12]; *Dinnes 2021* [13]; *Lee 2021* [14]; *Yoon 2021* [15].

- The above systematic reviews included all studies assessing the diagnostic effectiveness of NEAR test versus the RT-PCR method, including studies, in which the NEAR test **was used according to out-of-date protocol**. This means that research where: samples were diluted in UTM (Universal Transport Medium); samples were taken from two different anatomical sites; samples were stored for 48h and/or frozen, were included.
- The results obtained in this SLR differ from those reported in other systematic reviews. In the identified SLRs, the sensitivity of the NEAR test compared with the RT-PCR method ranged from 0.73 to 0.797, compared with 0.956-0.987 in presented analysis. The specificity of the NEAR test compared with the RT-PCR method ranged from 0.99 to 1.00 in the identified SLRs, consistent with our analysis (0.995 - 0.989). The main reason for this discrepancy in the sensitivity of the NEAR test between our analysis and the identified SLRs was the eligibility criteria. Studies, which were not in line with the currently recommended protocol for the use of the NEAR diagnostic test, were excluded from this SLR, as they could not provide valid information on how the test works if it is used in proper conditions. **This data confirms that the introduction of the new NEAR application protocol has significantly improved the sensitivity of the test.**

Table 5. Comparison of the present SLR with other identified SLRs

Author year		Sensitivity	Specificity	Dilution in UTM	Swabs from different anatomical sites	Samples stored for >48h or/and frozen	No of included studies not in line with the current protocol
Present SLR	Base case analysis	0.956	0.995	No	No	No	0/7
	Sensitivity analysis 1*	0.987	0.989				
Subsoontorn 2020 [11]		0.74	0.99	Yes	Yes	Yes	5/5
Van Walle 2020 [12]		0.797	NA	Yes	Yes	Yes	5/6
Dinnes 2021 [13]		0.73	0.997	Yes	Yes	Yes	10/11
Lee 2020 [14]		0.79	1.00	Yes	Yes	Yes	8/10
Yoon 2021 [15]		0.78	1.00	Yes	Yes	Yes	6/7

NA – not available; * closest to compliance with the protocol

Discussion

Key findings

- A meta-analysis of prospective studies in symptomatic patients found that the sensitivity of NEAR test for the diagnosis of acute infectious respiratory disease due to SARS-CoV-2 infection in symptomatic patients was **0.956** (95% CI: 0.918-0.976) and the specificity was **0.995** (95% CI: 0.946-0.999) compared with the RT-PCR method.
- The highest sensitivity of NEAR test was in symptomatic patients in whom no more than 7 days had elapsed since the onset of symptoms (population closest to compliance with the protocol), the sensitivity of NEAR test for the diagnosis of COVID -19 infection was **0.987** (95% CI: 0.917-0.998) and the specificity was **0.989** (95% CI: 0.989-0.989) compared with the RT-PCR method.
- The other systematic reviews identified included studies in which the NEAR test was not performed according to the manufacturer's currently recommended protocol, which significantly undermined the effectiveness of the test.
- Use of NEAR test in accordance with the currently valid protocol provides optimal diagnostic accuracy, as demonstrated by the results of this systematic review of primary studies, which included only studies in which the NEAR test was used correctly.

Study limitations

- Although some studies did not provide full information on the symptoms duration or time between sampling and testing, the authors of this systematic review decided to consider them in the meta-analysis, as other requirements of intervention usage were met. Therefore, meta-analysis embraced broader population than strictly indicated in the manufacturer's guidance, which might be reflected in the real clinical practice, where some information might be missing as well. In order to verify evidence gaps in the studies sensitivity analyses were performed.
- Conservative inclusion criteria were applied, potentially undermining the effectiveness of NEAR test. This is shown by the results of sensitivity analysis 1, which reflects intervention protocol guidance the best.

Study strengths

- Robust, transparent and reproducible methodology in accordance with PRISMA statement.
- Inclusion of studies only adhering to the protocol recommended by the manufacturer

Conclusion

- There is a great unmet need for a rapid and sensitive diagnostic test for the detection of COVID -19. Currently, the long wait for test results from RT-PCR disrupts the work of hospitals and forces patients to wait many hours before being admitted to the ward. This unmet need could be met by providing access to rapid yet sensitive molecular diagnostic tests. One such test is the molecular test based on NEAR method.
- Use of NEAR diagnostic test in accordance with the manufacturer's protocol provides optimal diagnostic accuracy, as demonstrated by the results of our review of primary studies, which included only studies in which NEAR test was used correctly.

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