Systematic review

Diagnostic accuracy of rapid nucleic acid tests for group A streptococcal pharyngitis: systematic review and meta-analysis

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ABSTRACT

Background: Acute pharyngitis is one of the most common conditions in outpatient settings and an important source of inappropriate antibiotic prescribing. Rapid antigen detection tests (RADTs) offer diagnosis of group A streptococcus at the point of care but have limited sensitivity. Rapid nucleic acid tests (RNATs) are now available; a systematic review of their accuracy is lacking.
Objectives: To evaluate the accuracy of RNATs in patients with pharyngitis; to explore test-level and study-level factors that could explain variability in accuracy; and to compare the accuracy of RNATs with that of RADTs.
Data sources: MEDLINE, Embase, Web of Science (1990–2020).
Study eligibility criteria: Cross-sectional studies and randomized trials.
Participants: Patients with pharyngitis.
Index test/s and reference standards: RNAT commercial kits compared with throat culture.
Methods: We assessed risk of bias and applicability using QUADAS-2. We performed meta-analysis of sensitivity and specificity using the bivariate random-effects model. Variability was explored by sub-group analyses and meta-regression.
Results: We included 38 studies (46 test evaluations; 17 411 test results). RNATs were most often performed in a laboratory. The overall methodological quality of primary studies was uncertain because of incomplete reporting. RNATs had a summary sensitivity of 97.5% (95% CI 96.2%–98.3%) and a summary specificity of 95.1% (95% CI 93.6%–96.3%). There was low variability in estimates across studies. Variability in sensitivity and specificity was partially explained by test type (p < 0.05 for both). Sensitivity analyses limited to studies with low risk of bias showed robust accuracy estimates. RNATs were more sensitive than RADTs (13 studies; 96.8% versus 82.3%, p 0.004); there was no difference in specificity (p 0.92).
Conclusions: The high diagnostic accuracy of RNATs may allow their use as stand-alone tests to diagnose group A streptococcus pharyngitis. Based on direct comparisons, RNATs have greater sensitivity than RADTs and equal specificity. Further studies should evaluate RNATs in point-of-care settings.
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Introduction

Acute pharyngitis is responsible for 15 million visits to physicians each year in the USA [1]. The most common bacterial cause of pharyngitis is group A streptococcus (GAS), accounting for 5%–15% of cases of pharyngitis in adults and 20%–40% in children [1,2]. GAS pharyngitis is usually mild and self-limiting, but a minority of patients might develop suppurative or non-suppurative complications. Antimicrobial therapy may prevent some of these complications and reduce the duration of symptoms and spread of GAS to others [3]. In high-income countries, non-suppurative post-streptococcal diseases have almost disappeared [4], and the public health goal is now to contain antimicrobial resistance by reducing the inappropriate use of antibiotics. These are prescribed in about 60% of visits for sore throat in adults [5] and in children [5,6], which is much higher than expected according to the proportion of cases due to GAS.

Because the signs and symptoms of GAS and viral pharyngitis broadly overlap [1], clinical scoring systems [7] (e.g. Centor score [8]) are not sufficiently accurate for clinical decision-making. Hence, most international clinical practice guidelines recommend diagnosing GAS by microbiological means [9]. The most accepted reference standard for GAS pharyngitis is throat culture on a blood agar plate, performed in a microbiology laboratory [10]. The advantages of throat culture are its ability to detect bacteria other than GAS and to perform antimicrobial susceptibility testing. The major limitation is the 24- to 48-hour delay in obtaining results. Rapid antigen detection tests (RADTs) were developed in the 1980s and are now widely used by clinicians [11]. They can provide results within 10 minutes and can be performed at the point of care (POC). RADTs are highly specific (about 95%) but usually have relatively low (around 85%) and highly variable (from 70% to 99%) sensitivity [12,13]. As such, several clinical practice guidelines recommend backing-up negative RADT results with a throat culture.

More recently, molecular tests that detect GAS-specific genes, such as the streptococcal pyrogenic exotoxin B (*speB*) gene sequence, have been developed [14]. Some rapid nucleic acid tests (RNATs) can be performed within 15 minutes. Like RADTs, RNATs provide binary results. There are different types of RNATs: single-stranded DNA probe assays (ssDNA) [15], real-time quantitative PCR (qPCR) [16], loop-mediated isothermal amplification (LAMP) [17], helicase-dependent amplification (HDA) [17] and nicking-enzyme amplification reaction (NEAR) [18]. RNATs seem to have high sensitivity and specificity [13], both around 95% [16,19], so potentially removing the need for backup culture. However, RNATs may require specialized and costly equipment, making them most suitable in outpatient clinics and hospital laboratories [20,21], rather than in physician offices. A recent systematic review and meta-analysis of the accuracy of RNATs is lacking.

The aims of the present study were to (a) evaluate the diagnostic accuracy of RNATs for GAS in patients with pharyngitis, (b) explore test-level and study-level factors that could explain variability in accuracy across studies and (c) compare the accuracy of RNATs with that of RADTs using culture as the reference standard.

Materials and methods

This study was registered in PROSPERO (CRD42020157289), conducted according to guidance from the Cochrane Handbook for systematic reviews of diagnostic test accuracy [22], and reported according to the PRISMA-DTA statement (see Supplementary material, Appendix S1) [23].

Eligibility criteria

We included cross-sectional studies reporting the diagnostic accuracy of one or more RNAT commercial kits for diagnosing GAS, with laboratory throat culture as the reference standard. We also included studies that directly compared the accuracy of one or more RADT(s), to the accuracy of one or more RNAT(s), within the same patients, using culture as the reference standard. Randomized controlled trials (RCTs) were eligible if we could extract 2 × 2 tables. We included studies of participants without any age limitation, seeking medical care because of a sore throat or with a diagnosis of pharyngitis, with or without evaluation of the likelihood of GAS origin (e.g. using a clinical scoring system such as the Centor score). Studies in any setting were eligible. We excluded studies in which only participants with a negative RADT result underwent an RNAT as an add-on test. We included studies of all types of commercially available RNATs (ssDNA, qPCR, LAMP, HDA and NEAR). To be considered as 'rapid', nucleic acid tests must be able to give a result within 2 hours after the throat sample was taken [24]. For studies involving more than one throat culture technique (e.g. different medium), we *a priori* chose to extract data related to a simple blood agar plate with a total of 48 hours of incubation in an aerobic atmosphere, as recommended [10].

Literature searches and study selection

We searched MEDLINE, Web of Science and Embase on 4 November 2020, using the search strategy described in the Supplementary material (Appendix S2). We did not use any filter related to age or language, nor any methodological filters [25]. The searches were run from 1990 onwards because RNATs were not available before this date. We also hand-searched reference lists of included primary studies and relevant review articles identified through the search, and the 20 first 'related articles' of each included article in PubMed. We used Google Scholar to identify reports that cited included primary studies. We also searched online resources of professional societies, conferences and international guidelines (see Supplementary material, Appendix S3). Two review authors (CD, JFC) independently excluded studies that were not related to pharyngitis or RNATs based on the titles and abstracts. Two review authors (CD, JFC) retrieved the full text of relevant articles and independently evaluated them for inclusion. All discrepancies were resolved with discussion. In cases of multiple reports for a given study or when we were not able to exclude the possibility of overlapping populations, we included the most recent report.

Data collection and quality assessment

For each included study, two review authors (CD, YR) independently extracted the number of true positives, true negatives, false positives and false negatives for each index test (see Supplementary material, Appendix S4). If such data were not provided, we calculated these numbers from the reported estimates of sensitivity and specificity, if available. For studies that used a third test for discrepant results between RNAT and culture results, we collected accuracy results only for RNAT versus culture, because discrepant analysis is likely to produce biased estimates [26]. If some data were unclear or missing, we attempted to contact the study authors to obtain additional information. For each study, two review authors (CD, YR) independently assessed risk of bias and concerns about applicability using the QUADAS-2 tool [27]. We tailored the quality assessment tool to our review question and developed specific guidance for scoring each item (see Supplementary material, Appendix S5). We extracted all

data using a prespecified form (see Supplementary material, Appendix S4).

Statistical analysis

We plotted individual study estimates of sensitivity and specificity on forest plots and in the receiver-operating characteristic space. Then, we used Stata/SE-16.0 (StataCorp, College Station, TX, USA) with 'metandi' and 'xtmelogit' [28] to fit the hierarchical bivariate model [29], which allows for calculating summary estimates of sensitivity and specificity and the associated 95% CI. We included the same study in the meta-analysis more than once if the study reported different index tests.

Variability in accuracy across studies was first assessed through visual inspection of the forest plots and receiver-operating characteristic space. Then, we used meta-regression to formally investigate sources of variability, by incorporating the following covariates in the bivariate model: test type (ssDNA, qPCR, LAMP, NEAR or HDA), age (adults versus children), GAS prevalence (dichotomized with a median split), and study setting (emergency room, walk-in clinic and physician's office, hospital and mixed setting (including schools)).

We also conducted a meta-regression analysis to compare the accuracy of RADTs and RNATs using direct head-to-head evidence (i.e. we restricted this analysis to studies that evaluated both test types in the same participants using culture as reference standard) [22,30]; when studies evaluated several RNATs or several RADTs, we randomly selected one test for each type to avoid the use of a single evaluation in multiple test comparisons.

To assess the robustness of our findings, we carried out sensitivity analyses restricted to: (a) studies judged at low risk of bias in at least two QUADAS-2 domains, (b) studies judged to have low applicability concerns in at least two QUADAS-2 domains, (c) studies evaluating RNATs with a nucleic acid amplification step (i.e. excluding studies assessing ssDNA-based tests such as Gen-Probe® Group A Streptococcus Direct Test), and (d) RNATs with low complexity (i.e. Clinical Laboratory Improvement Amendments (CLIA)-waived or requiring no more than two pipetting steps). We did not try to assess reporting bias [23].

Results

Results of the search

From 3176 records screened, a total of 38 unique studies were included (Fig. 1, see Supplementary material, Appendix S6) [15,16,19,20,31−52], including 12 unpublished studies [53−64]. All unpublished studies were abstracts and posters presented at conferences. Of the 12 unpublished studies, nine were identified in Embase. The three others were found through an extensive search of conference abstracts, as described in the Supplementary material (Appendix S3). Five studies [20,31,43,53,59] evaluated several RNATs, resulting in a total of 46 test evaluations, encompassing a total of 17 411 test results (from 16 039 individual patients), of which 3763 (22%) returned positive for GAS according to culture.

Study characteristics

The main characteristics of the included studies are detailed in Table 1. Most studies were conducted in the USA (31/38, 82%). Twelve different commercial RNAT kits targeting six different GAS genes (*speB*, *sdaB*, *cepA*, *pts1*, *spy1258*, rRNA subunits) were evaluated (see Supplementary material, Appendix S7). Twenty-one evaluations (6312 test results) assessed a test relying on qPCR technology, seven evaluations (4043 test results) ssDNA, six evaluations (2595 test results) LAMP, seven evaluations (3393 test results) HDA, and five evaluations (1068 test results) NEAR. The most frequent clinical setting was walk-in clinics (10/38, 26%). Tests were performed directly at the POC, by non-expert personnel, in only three studies [36,44,51], although in the majority (35/38, 92%), RNATs were performed in a laboratory. No study provided information on whether RNAT results were given to clinicians to evaluate their impact on patient management. The median number of participants per evaluation was 266 (interquartile range 160−520). The median of GAS prevalence by throat culture was 27% (interquartile range 20%−33%). Fifteen studies (39%) included both adults and children, whereas 12 (32%) included only children; the remaining 11 (29%) studies did not give any information on patient age. At least 6736/17 411 (39%) test results were from children.
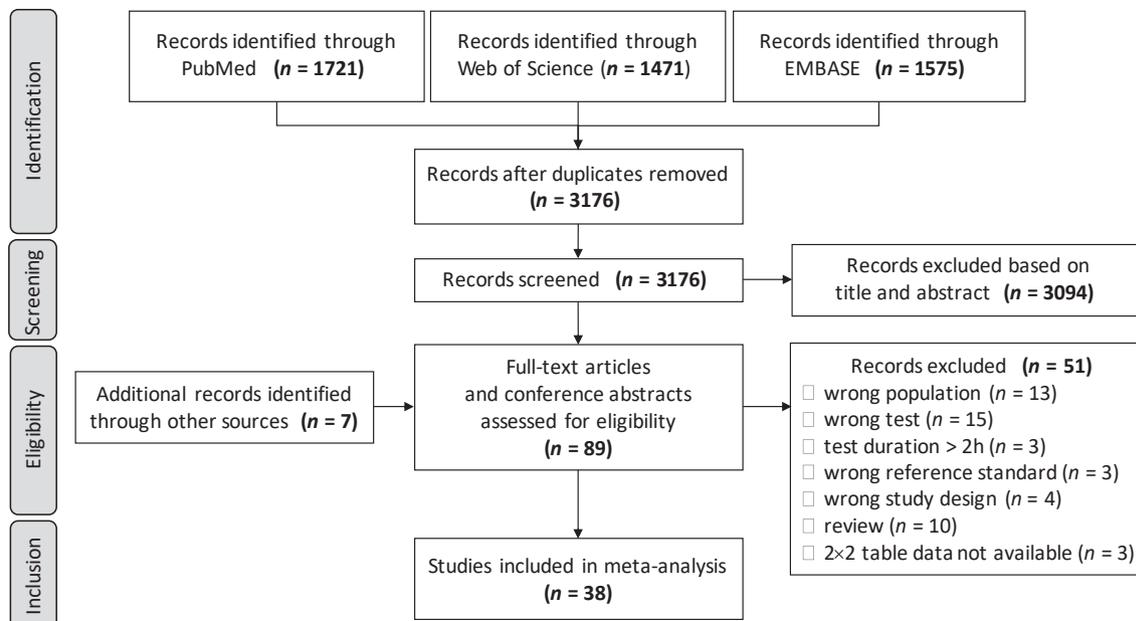

Fig. 1. Flow diagram of studies in the review.

Table 1
Characteristics of included studies (n = 38)

Author (year) [Ref]	Data collection	Patient selection	Clinical selection	Population	Country	Clinical setting	Test setting	Reference standard	Type of RNAT(s)	Commercial kit (target gene)	Comparison with a RADT	GAS prevalence,[a] %	No. of participants
Amrud (2019) [31]	NR	NR	NR	Adults and children	Canada	Hospital	Laboratory	Throat culture on a BAP during 24 h	qPCR, qPCR, HDA	Aries (sdaB), Simplexa (speB), Solana (sdaB)	No	40.9	287 (858 test results)
Anderson (2013) [32]	Pro	NR	None	Adults and children	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	LAMP	Illumigene (speB)	No	9.3	796
Banerjee (2020) [33]	Pro	NR	NR	Adults and children	USA and Canada	NR	Laboratory	Throat culture on a BAP during >48 h	qPCR	GenePOC (NR)	No	25.5	604
Beck (2018) [53]	Pro	NR	NR	NR	USA	NR	Laboratory	Throat culture	NEAR, qPCR, qPCR	Alere i (cepA), Cobas Liat (spy1258), Xpert Xpress (speB)	Yes	28.9	194 (582 test results)
Berry (2018) [34]	Pro	NR	NR	Adults and children	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	NEAR	Alere i (cepA)	Yes	19.5	216
Bourbeau (2004) [35]	Pro	NR	NR	NR	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	ssDNA	Gen-Probe (rRNA subunits)	No	21.7	1005
Chan (2013) [54]	NR	NR	NR	Adults and children	Not reported	Mixed	Laboratory	Throat culture on a BAP during 48 h	LAMP	Illumigene (speB)	No	3.1	196
Chapin (2002) [19]	Pro	Consecutive	NR	Children	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	ssDNA	Gen-Probe (rRNA subunits)	Yes	33.1	520
Cheng (2012) [55]	NR	NR	NR	NR	USA	NR	Laboratory	Throat culture	qPCR	Simplexa (speB)	No	18.1	105
Cheng (2014) [56]	NR	NR	NR	NR	USA	NR	Laboratory	Throat culture	qPCR	Simplexa (speB)	No	37.9	87
Cohen (2015) [36]	Pro	NR	None	Adults and children	USA	Mixed	Point of care	Throat culture on a BAP during 48 h	NEAR	Alere i (cepA)	No	30.6	481
Eldirdiri (2019) [57]	Retro	NR	NR	Adults and children	UK	Mixed	Laboratory	Throat culture on a BAP during 48 h	qPCR	Xpert Xpress (speB)	No	6.4	323
Faron (2015) [37]	Pro	NR	NR	NR	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	HDA	AmpliVue (sdaB)	No	14.6	1192
Felsenstein (2014) [38]	Pro	Consecutive	None	Children	USA	Emergency room	Laboratory	Throat culture on a BAP during 48 h	LAMP	Illumigene (speB)	Yes	16.1	361
Fox (2006) [39]	Pro	NR	None	Children	USA	Emergency room	Laboratory	Throat culture on a BAP during 48 h	ssDNA	Gen-Probe (rRNA subunits)	Yes	56.6	53
Gregson (2015) [58]	Retro	NR	NR	NR	USA	NR	Laboratory	Throat culture	qPCR	Simplexa (speB)	No	15.6	655
Heelan (1996) [40]	Pro	NR	NR	NR	USA	Mixed	Laboratory	Throat culture on a BAP during 48 h	ssDNA	Gen-Probe (rRNA subunits)	No	23.9	318
Heiter (1993) [15]	Pro	NR	NR	Adults and children	USA	Mixed	Laboratory	Throat culture on a BAP during 48 h	ssDNA	Gen-Probe (rRNA subunits)	Yes	23.7	1103
Henson (2013) [41]	Pro	Consecutive	NR	Children	USA	Hospital	Laboratory	Throat culture on a BAP	LAMP	Illumigene (speB)	Yes	21.1	437
Ivaska (2017) [59]	Pro	Convenience	None	Children	Finland	Emergency room	Laboratory	Throat culture on a BAP during 48 h	LAMP, qPCR	Illumigene (speB), Simplexa (speB)	Yes	22.9	83 (166 test results)
Kanwar (2018) [42]	Pro	NR	None	Adults and children	USA	Mixed	Laboratory	Throat culture on a BAP during 48 h	qPCR	Aries (sdaB)	No	25.9	623
Parker (2019) [43]	Pro	Convenience	NR	Adults and children	USA	Mixed	Laboratory	Throat culture on a BAP during 48 h	qPCR, qPCR, qPCR	Aries (sdaB), Cobas Liat (Spy1258), Xpert Xpress (speB)	Yes	42.6	68 (178 test results)
Pickering (2020) [44]	Pro	Consecutive	None	Children	Australia	Primary school	Point of care	Throat culture on a BAP	NEAR	Alere i (cepA)	No	50.0	18
Pierce (2014)	NR	Convenience	NR	Children	USA	Walk-in clinic	Laboratory	Throat culture	HDA	AmpliVue (sdaB)	No	22.9	204
Pierce (2015) [60]	Pro	Convenience	NR	Children	USA	Hospital	Laboratory	Throat culture	HDA	Solana (sdaB)	No	32.4	213
Pierce (2016) [61]	Pro	Convenience	NR	Children	USA	Hospital	Laboratory	Throat culture	HDA	Solana (sdaB)	No	27.3	176
Pokorski (1994) [45]	Pro	NR	NR	NR	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during >48 h	ssDNA	Gen-Probe (rRNA subunits)	No	11.9	767
Purcell (2018) [63]	Pro	NR	NR	NR	USA	NR	Laboratory	Throat culture	qPCR	XCR NeuMoDx (NR)	No	50.0	80
Ralph (2019) [46]	Pro	NR	NR		Australia	Hospital	Laboratory	Throat culture	qPCR	Xpert Xpress (speB)	No	30.6	62

Table 1 (continued)

Author (year) [Ref]	Data collection selection	Patient selection	Clinical selection	Population	Country	Clinical setting	Test setting	Reference standard	Type of RNAT(s)	Commercial kit (target gene)	Comparison with a RADT	GAS prevalence,[a] %	No. of participants
Rao (2019) [20]	Pro	Consecutive	None	Adults and children	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	qPCR, HDA	Cobas Liat (Spy1258), Solana (sdaB)	Yes	31.1	275 (550 test results)
Selvarangan (2017) [64]	Pro	NR	NR	Children	USA	NR	Laboratory	Throat culture on a BAP during 48 h	qPCR	Aries (sdaB)	No	27.7	184
Steed (1993) [47]	Pro	NR	NR	NR	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during >48 h	ssDNA	Gen-Probe (rRNA subunits)	No	21.3	277
Tabb (2016) [48]	Pro	NR	NR	Adults and children	USA	NR	Laboratory	Throat culture	qPCR	Simplexa (speB)	No	11.5	1352
Uhl (2003) [16]	Pro	Consecutive	NR	Adults and children	USA	Walk-in clinic	Laboratory	Throat culture on a BAP during 48 h	qPCR	LightCycler (pts1)	Yes	14.3	423
Uphoff (2016) [49]	Pro	NR	NR	Adults and children	USA	Mixed	Laboratory	Throat culture on a BAP during 48 h	HDA	Solana (sdaB)	No	20.7	1082
Upton (2016) [50]	Pro	NR	None	Children	New Zealand	Primary school	Laboratory	Throat culture on a BAP during 48 h	LAMP	Illumigene (speB)	No	12.2	757
Wang (2017) [51]	Pro	NR	Explicit criteria	Adults and children	USA	Physician's office	Point of care	Throat culture	qPCR	Cobas Liat (Spy1258)	Yes	30.2	427
Weinzierl (2018) [52]	Pro	NR	NR	Children	USA	Emergency room	Laboratory	Throat culture on a BAP during 48 h	NEAR	Alere i (cepA)	Yes	38.1	160

Abbreviations: BAP, blood agar plate; GAS, group A streptococcus; HDA, helicase-dependent amplification; LAMP, loop-mediated isothermal amplification; NEAR, nicking enzyme amplification reaction; NR, not reported; qPCR, quantitative (real-time) polymerase chain reaction; Pro, prospective; Retro, retrospective; RNAT, rapid nucleic acid test; RADT, rapid antigen detection test; ssDNA, single-stranded DNA.

[a] GAS prevalence according to the reference standard (throat culture).

Thirteen studies [15,16,19,20,34,38,39,41,43,51−53,59] directly compared an RNAT against an RADT.

Risk of bias and applicability

The overall methodological quality of included studies is summarized in Fig. 2; the assessment of individual studies is shown in the Supplementary material (Appendix S8). All studies were cross-sectional. The majority of studies (27/38, 71%) did not clearly report whether participants formed a consecutive, random, or convenience series. Symptoms for inclusion were described in eight studies (21%). Nine studies (24%) avoided clinical selection of participants by including all patients with pharyngitis regardless of their clinical severity (e.g. regardless of the participants' Centor scores), whereas one study selected patients on explicit criteria; 28 (74%) studies did not give enough details to judge whether the study avoided such clinical selection. Interpretation of the results of the RNAT was made with blinding of the result of throat culture in one-third of the studies (13/38, 34%). An appropriate reference standard (i.e. laboratory throat culture on a blood agar plate for 48 hours) was used in 24 studies (63%). Interpretation of the results of the reference standard was made with blinding of the result of the RNAT in eight studies (21%). The main reasons for scoring the flow and timing domain as 'high risk of bias' were the lack of report of withdrawals (25/38 studies), lack of report of uninterpretable/intermediate test results (21/38 studies) and an inappropriate (i.e. >48 h) time interval between RNAT and culture (16/38 studies).

Diagnostic accuracy of rapid nucleic acid tests

Across the 46 evaluations included in the quantitative synthesis, RNAT sensitivity ranged from 82% to 100%, and specificity from 56% to 100% (Fig. 3). When data were combined in meta-analysis, RNATs had a summary sensitivity of 97.5% (95% CI 96.2%−98.3%) and a summary specificity of 95.1% (93.6%−96.3%; Fig. 4a).

Visual inspection of the forest plots and receiver-operating characteristic space suggested low variability in accuracy estimates across studies. Two small studies [44,46] were outliers regarding specificity (specificities 56%, 95% CI 21%−86% and 70%, 95% CI 54%−83%, respectively). Both were conducted in Australia in specific settings (Table 1). In a sensitivity analysis excluding these two outlier studies with low RNAT specificity, meta-analysis estimates were not affected: sensitivity 97.3% (96.0%−98.2%) and specificity 95.5% (94.2%−96.5%).

The results of statistical investigations of variability are summarized in Table 2. As no study included adults only, we could not perform a meta-regression on age group. There was statistical evidence that sensitivity and specificity differed between test types (p < 0.001 and p 0.038, respectively), with ssDNA tests having the lowest sensitivity (90.5%, 95% CI 84.7%−94.3%) and HDA tests the lowest specificity (92.7%, 95% CI 87.4%−95.9%). RNAT sensitivity was significantly higher in studies with GAS prevalence above the median compared with studies below the median (98.5%, 95% CI 97.3%−99.2% versus 95.9%, 95% CI 93.5%−97.4%, respectively; p 0.007). Specificity did not statistically depend on prevalence (p 0.448). Meta-regression according to study setting identified significant variations in sensitivity (p 0.047), the lowest estimate coming from emergency room settings (summary sensitivity 95.1%, 95% CI 86.6%−98.3%); specificity seemed lower in studies conducted in hospital settings (summary specificity 90.0%, 95% CI 82.5%−94.5%), but the difference did not reach statistical significance (p 0.136). The results of sensitivity analyses are presented in Table 2. Compared with the overall results, accuracy estimates were robust in all sensitivity analyses.
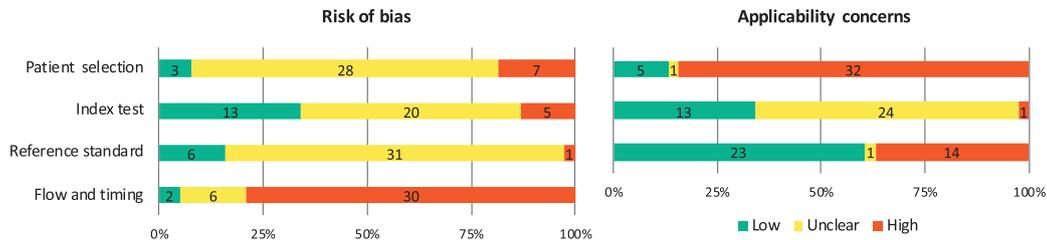
Fig. 2. Risk of bias and applicability concerns graph: review authors' judgements about each domain across all included studies (*n* = 38).

Comparative accuracy of rapid nucleic acid tests against rapid antigen detection tests

Thirteen studies directly compared RNATs to RADTs (4224 patients; Fig. 4b). The sensitivity of RNATs was higher than that of RADTs (96.8%, 95% CI 94.6%−98.1% versus 82.3%, 95% CI 65.0%−92.1%; p 0.004) whereas specificity was not different (97.0%, 95% CI 94.3%−98.5% versus 97.2%, 95% CI 94.3%−98.6%; p 0.92). Of note, there was an outlier within the RADT group, with one small study (*n* = 51) reporting an RADT sensitivity of 5.3%. A sensitivity analysis excluding this study yielded similar estimates of RADT sensitivity (85.5%, 95% CI 74.8%−92.1%) and specificity (97.0%, 95% CI 93.9%−98.6%).

Discussion

Main findings

In this systematic review, we included 46 evaluations with 17 411 test results (from 16 039 individual participants) that evaluated RNATs for detecting GAS in patients with pharyngitis. The overall methodological quality of the included studies was difficult to assess because of incomplete reporting; this resulted in a majority of 'unclear' risk-of-bias judgements for patient selection, index test and reference standard QUADAS-2 domains. The summary estimates of RNAT sensitivity and specificity were 97.5% (95% CI 96.2%−98.3%) and 95.1% (95% CI 93.6%−96.3%), respectively. Overall, there was little variability in sensitivity and specificity across studies. RNAT sensitivity was significantly associated with test type, prevalence and study setting. The association between GAS prevalence and rapid test sensitivity has already been described [65]: high prevalence might reflect the inclusion of patients with more severe signs and symptoms (e.g. higher Centor score), which correlates with higher bacterial inoculum. RNAT specificity was not affected by GAS prevalence, maybe because RNATs use a selection of molecular primers that are designed for avoiding cross-reaction with other bacterial species. Summary accuracy estimates were robust in sensitivity analyses. When directly compared with RADTs, RNATs had significantly higher sensitivity (96.8% versus 82.3%).

Implications

The role of RNATs will depend on local epidemiology, clinical needs and available resources (see possible clinical pathways for GAS testing in people with pharyngitis in the Supplementary material, Appendix S9). The high sensitivity and specificity of RNATs suggest that they could replace throat culture in microbiology laboratories and become a new reference standard for GAS pharyngitis. However, contrary to culture, RNATs would not provide information on antimicrobial resistance patterns, and the identification would be limited to GAS, so ignoring other pathogens such as Group C and Group G streptococci. The use of RNATs as add-on tests

after a negative RADT result was evaluated in three studies [66−68], which were not within the scope of this review. With reported sensitivities between 91.4% and 100%, and specificities between 89% and 100%, these studies might also support replacing backup culture with an RNAT in case of a negative RADT result.

Whether RNATs could replace RADTs as first-line tests at the POC remains controversial. Evidence shows that more sensitive rapid tests like RNATs could decrease antibiotic prescription [20,69]. The rapidity of results availability is crucial, because a significant proportion of clinicians usually do not wait for culture results to prescribe antibiotics or do not stop treatment after obtaining negative culture results [70]. Also, the limited sensitivity of RADTs might drive clinicians to distrust negative test results, therefore prescribing antibiotics even in the face of a negative RADT result, because they do not want to wait for backup culture results [69]. However, because of their higher sensitivity, RNATs may be counter-productive by increasing antibiotic prescription rates [71]. RNATs could lead to overdiagnosis because they may detect more healthy carriers. Indeed, approximately 5%−20% of school-age children are GAS carriers [2]: they harbour GAS in their pharynx without symptoms, and they should not receive antibiotic treatment. RNATs may also detect patients with non-viable bacteria and very low bacterial counts in whom antibiotics are unnecessary [72]. We need to get a clearer view of the target range of the sensitivity and specificity needed to avoid antibiotic over-prescription issues while minimizing the risk of complications and disease spread [73]. A way to better align the sensitivity of RNATs with current clinical needs could be to test saliva samples instead of pharyngeal throat swabs. In a small pilot study (*n* = 20), 19 of 20 GAS-positive samples were also positive using an RNAT performed on saliva [74]. Another approach could be to increase the threshold for test positivity. For example, for qPCR-based tests, the cycle threshold (Ct) used to define test positivity is crucial and could be modified to obtain a lower test sensitivity [75].

Complexity and hands-on time of RNATs should also be considered (see Supplementary material, Appendix S7). Some commercial kits included in our review are technically complex, with several pipetting and vortexing/incubating steps. Such RNATs might not be routinely feasible in POC settings. However, we could not formally explore whether the test setting (laboratory versus POC) could be a source of variability in accuracy because RNATs were performed at the POC in only three studies, which is insufficient for the bivariate model to converge. Moreover, rapid tests may have lower performances when performed by clinicians at the POC [76]. For RNATs that take too long for patients to wait for the result, it was proposed that the clinician could send the patient home and transmit an antibiotic prescription to the patient's retail pharmacist only if the result of the RNAT was positive [21].

Another drawback of RNATs is their cost. Although the price of RNATs may decrease over time with wider clinical use, RNATs remain about ten times more expensive than RADTs (i.e. $10−$50 per unit and $1−$5 per unit for RNATs and RADTs, respectively). The cost-effectiveness of RNATs should be evaluated by accounting

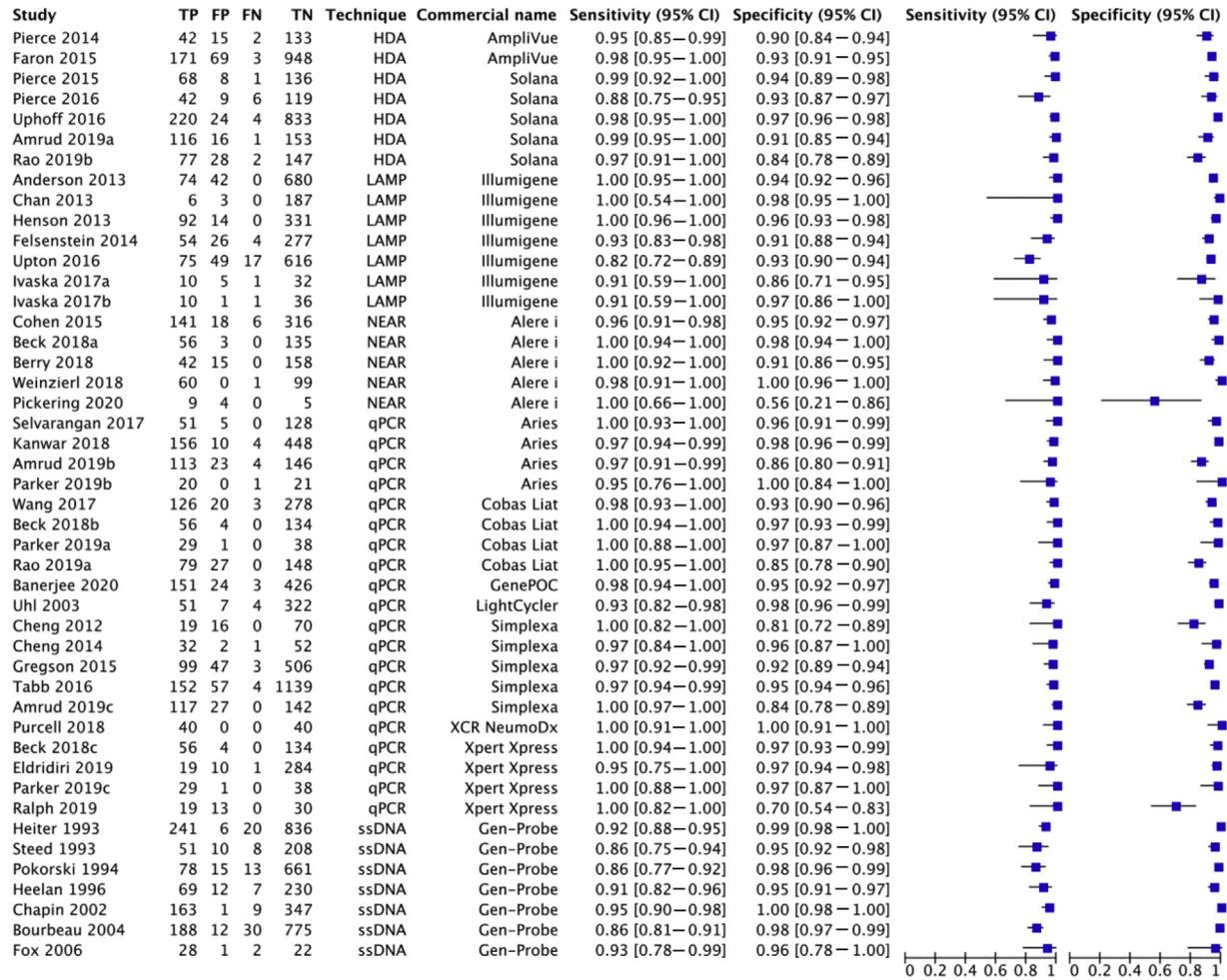| Study	TP	FP	FN	TN	Technique	Commercial name	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Pierce 2014	42	15	2	133	HDA	AmpliVue	0.95 [0.85–0.99]	0.90 [0.84–0.94]		
Faron 2015	171	69	3	948	HDA	AmpliVue	0.98 [0.95–1.00]	0.93 [0.91–0.95]		
Pierce 2015	68	8	1	136	HDA	Solana	0.99 [0.92–1.00]	0.94 [0.89–0.98]		
Pierce 2016	42	9	6	119	HDA	Solana	0.88 [0.75–0.95]	0.93 [0.87–0.97]		
Uphoff 2016	220	24	4	833	HDA	Solana	0.98 [0.95–1.00]	0.97 [0.96–0.98]		
Amrud 2019a	116	16	1	153	HDA	Solana	0.99 [0.95–1.00]	0.91 [0.85–0.94]		
Rao 2019b	77	28	2	147	HDA	Solana	0.97 [0.91–1.00]	0.84 [0.78–0.89]		
Anderson 2013	74	42	0	680	LAMP	Illumigene	1.00 [0.95–1.00]	0.94 [0.92–0.96]		
Chan 2013	6	3	0	187	LAMP	Illumigene	1.00 [0.54–1.00]	0.98 [0.95–1.00]		
Henson 2013	92	14	0	331	LAMP	Illumigene	1.00 [0.96–1.00]	0.96 [0.93–0.98]		
Felsenstein 2014	54	26	4	277	LAMP	Illumigene	0.93 [0.83–0.98]	0.91 [0.88–0.94]		
Upton 2016	75	49	17	616	LAMP	Illumigene	0.82 [0.72–0.89]	0.93 [0.90–0.94]		
Ivaska 2017a	10	5	1	32	LAMP	Illumigene	0.91 [0.59–1.00]	0.86 [0.71–0.95]		
Ivaska 2017b	10	1	1	36	LAMP	Illumigene	0.91 [0.59–1.00]	0.97 [0.86–1.00]		
Cohen 2015	141	18	6	316	NEAR	Alere i	0.96 [0.91–0.98]	0.95 [0.92–0.97]		
Beck 2018a	56	3	0	135	NEAR	Alere i	1.00 [0.94–1.00]	0.98 [0.94–1.00]		
Berry 2018	42	15	0	158	NEAR	Alere i	1.00 [0.92–1.00]	0.91 [0.86–0.95]		
Weinzierl 2018	60	0	1	99	NEAR	Alere i	0.98 [0.91–1.00]	1.00 [0.96–1.00]		
Pickering 2020	9	4	0	5	NEAR	Alere i	1.00 [0.66–1.00]	0.56 [0.21–0.86]		
Selvarangan 2017	51	5	0	128	qPCR	Aries	1.00 [0.93–1.00]	0.96 [0.91–0.99]		
Kanwar 2018	156	10	4	448	qPCR	Aries	0.97 [0.94–0.99]	0.98 [0.96–0.99]		
Amrud 2019b	113	23	4	146	qPCR	Aries	0.97 [0.91–0.99]	0.86 [0.80–0.91]		
Parker 2019b	20	0	1	21	qPCR	Aries	0.95 [0.76–1.00]	1.00 [0.84–1.00]		
Wang 2017	126	20	3	278	qPCR	Cobas Liat	0.98 [0.93–1.00]	0.93 [0.90–0.96]		
Beck 2018b	56	4	0	134	qPCR	Cobas Liat	1.00 [0.94–1.00]	0.97 [0.93–0.99]		
Parker 2019a	29	1	0	38	qPCR	Cobas Liat	1.00 [0.88–1.00]	0.97 [0.87–1.00]		
Rao 2019a	79	27	0	148	qPCR	Cobas Liat	1.00 [0.95–1.00]	0.85 [0.78–0.90]		
Banerjee 2020	151	24	3	426	qPCR	GenePOC	0.98 [0.94–1.00]	0.95 [0.92–0.97]		
Uhl 2003	51	7	4	322	qPCR	LightCycler	0.93 [0.82–0.98]	0.98 [0.96–0.99]		
Cheng 2012	19	16	0	70	qPCR	Simplexa	1.00 [0.82–1.00]	0.81 [0.72–0.89]		
Cheng 2014	32	2	1	52	qPCR	Simplexa	0.97 [0.84–1.00]	0.96 [0.87–1.00]		
Gregson 2015	99	47	3	506	qPCR	Simplexa	0.97 [0.92–0.99]	0.92 [0.89–0.94]		
Tabb 2016	152	57	4	1139	qPCR	Simplexa	0.97 [0.94–0.99]	0.95 [0.94–0.96]		
Amrud 2019c	117	27	0	142	qPCR	Simplexa	1.00 [0.97–1.00]	0.84 [0.78–0.89]		
Purcell 2018	40	0	0	40	qPCR	XCR NeumoDx	1.00 [0.91–1.00]	1.00 [0.91–1.00]		
Beck 2018c	56	4	0	134	qPCR	Xpert Xpress	1.00 [0.94–1.00]	0.97 [0.93–0.99]		
Eldridiri 2019	19	10	1	284	qPCR	Xpert Xpress	0.95 [0.75–1.00]	0.97 [0.94–0.98]		
Parker 2019c	29	1	0	38	qPCR	Xpert Xpress	1.00 [0.88–1.00]	0.97 [0.87–1.00]		
Ralph 2019	19	13	0	30	qPCR	Xpert Xpress	1.00 [0.82–1.00]	0.70 [0.54–0.83]		
Heiter 1993	241	6	20	836	ssDNA	Gen-Probe	0.92 [0.88–0.95]	0.99 [0.98–1.00]		
Steed 1993	51	10	8	208	ssDNA	Gen-Probe	0.86 [0.75–0.94]	0.95 [0.92–0.98]		
Pokorski 1994	78	15	13	661	ssDNA	Gen-Probe	0.86 [0.77–0.92]	0.98 [0.96–0.99]		
Heelan 1996	69	12	7	230	ssDNA	Gen-Probe	0.91 [0.82–0.96]	0.95 [0.91–0.97]		
Chapin 2002	163	1	9	347	ssDNA	Gen-Probe	0.95 [0.90–0.98]	1.00 [0.98–1.00]		
Bourbeau 2004	188	12	30	775	ssDNA	Gen-Probe	0.86 [0.81–0.91]	0.98 [0.97–0.99]		
Fox 2006	28	1	2	22	ssDNA	Gen-Probe	0.93 [0.78–0.99]	0.96 [0.78–1.00]		

Fig. 3. Forest plots of rapid nucleic acid tests sensitivity and specificity for group A streptococcus detection, ordered by technique and commercial kit (n = 46). Abbreviations: FN, false negative; FP, false positive; TN, true negative; TP, true positive.
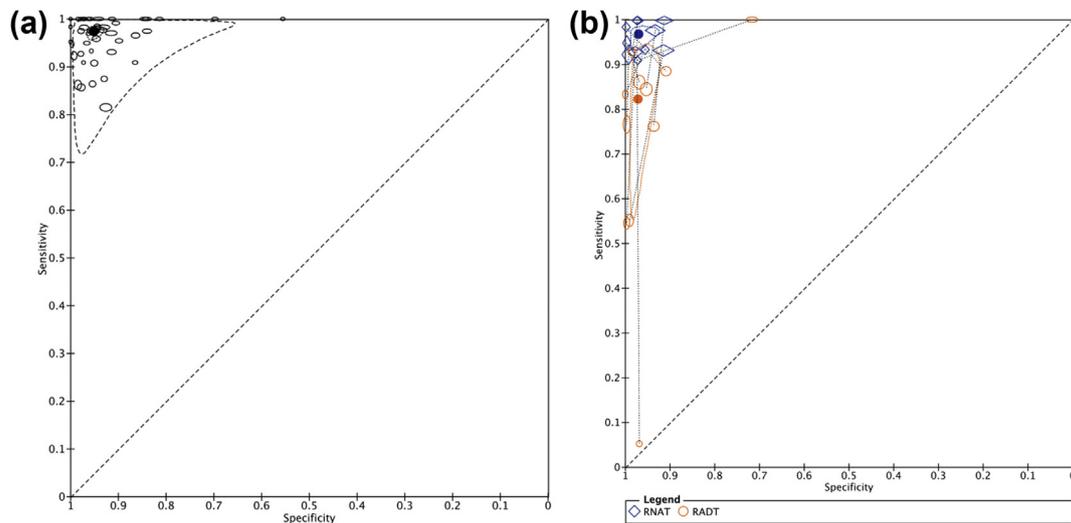

Fig. 4. Summary receiver-operating characteristic plots (a) All included evaluations (n = 46). Each individual evaluation is represented by an empty circle, with size proportional to the inverse standard error. The filled circle is the summary estimate for sensitivity and specificity. The narrow-dotted curve represents the 95% confidence region around the summary estimate; the wider dashed curve represents the 95% prediction region. (b) Direct comparison of rapid nucleic acid tests (RNAT) versus rapid antigen detection tests (RADT) (n = 13). Each evaluation is represented by an empty blue diamond (RNAT) and an empty orange circle (RADT), connected by a dotted line. The filled blue circle is the pooled summary estimate for sensitivity and specificity of RNATs; the filled orange circle is the pooled summary estimate for sensitivity and specificity of RADTs. The dotted curves represent the 95% confidence region around the summary estimate.

Table 2
Main meta-analysis, subgroup analyses with meta-regression, sensitivity analyses and comparison of rapid nucleic acid test (RNATs) versus rapid antigen detection tests (RADTs)

Analysis	Evaluations (*n*)	Test results (*n*)	Sensitivity, % (95% CI)	p	Specificity, % (95% CI)	p
Main meta-analysis	46	17 411	97.5 (96.2–98.3)		95.1 (93.6–96.3)	
Subgroup analysis: Test type						
qPCR	21	6312	98.3 (97.2–99.0)	<0.001	94.2 (91.7–96.0)	0.038
ssDNA	7	4043	90.5 (84.7–94.3)		98.2 (96.5–99.1)	
LAMP	6	2595	95.6 (90.5–98.1)		95.3 (91.1–97.6)	
HDA	7	3393	97.5 (95.1–98.7)		92.7 (87.4–95.9)	
NEAR	5	1068	98.4 (95.4–99.5)		94.8 (88.9–97.6)	
Subgroup analysis: GAS prevalence[a]						
Below the median	23	12 824	95.9 (93.5–97.4)	0.007	95.6 (93.7–97.0)	0.448
Above the median	23	4587	98.5 (97.3–99.2)		94.6 (91.9–96.4)	
Subgroup analysis: Study setting						
Emergency room	5	670	95.1 (86.6–98.3)	0.047	95.6 (89.5–98.2)	0.136
Walk-in clinic and physician's office	12	6282	96.3 (93.2–98.0)		95.1 (92.3–97.0)	
Hospital	7	1746	98.5 (96.1–99.4)		90.0 (82.5–94.5)	
Mixed setting (including schools)	12	5064	96.1 (92.5–98.0)		96.6 (94.3–98.0)	
Not reported	10	3649	99.0 (97.5–99.6)		95.6 (92.4–97.5)	
Sensitivity analyses						
With ≥2 QUADAS-2 domains with low risk of bias	6	2531	96.5 (93.0–98.3)	–	93.4 (82.7–97.7)	–
With ≥2 QUADAS-2 domains with low applicability concerns	13	6592	96.5 (94.1–97.9)	–	96.9 (94.5–98.3)	–
RNATs with amplification (i.e. not ssDNA)	39	13 368	98.2 (97.1–98.8)	–	94.2 (92.5–95.6)	–
Low complexity RNATs	25	6996	98.2 (97.1–98.8)	–	94.2 (91.4–96.1)	–
Direct comparison: RNATs versus RADTs						
RNATs	13	4224	96.8 (94.6–98.1)	0.004	97.0 (94.3–98.5)	0.92
RADTs	13	3936	82.3 (65.0–92.1)		97.2 (94.3–98.6)	

Abbreviations: GAS, group A streptococcus; HDA, helicase-dependent amplification; LAMP, loop-mediated isothermal amplification; NEAR, nicking enzyme amplification reaction; qPCR, quantitative (real-time) polymerase chain reaction; RNAT, rapid nucleic acid test; RADT, rapid antigen detection test; ssDNA, single-stranded DNA.
  [a] Median GAS prevalence 27%.

for the cost of reagents, of backup tests [21], of false-positive results leading to adverse effects of antibiotics and increased antimicrobial resistance, and of false-negative results leading to untreated GAS complications.

Strengths and limitations

This study has strengths. These include using a broad range of complementary literature searches that allowed us to include 12 unpublished studies, independent and duplicate screening of records and data extraction, and pre-planned subgroup and sensitivity analyses.

Our review also has several limitations. First, incomplete reporting (compared with reporting standards for diagnostic accuracy studies such as STARD [77]) caused difficulties when evaluating the quality of studies and led to missing data for subgroup analyses and meta-regression. Many reports did not provide essential participant characteristics (e.g. age) and key study design features (e.g. symptoms for inclusion and blinding of test interpretation). Also, most studies did not mention whether participants were selected on their level of clinical severity (e.g. using the Centor score), so we cannot exclude selection bias. As a result, the included studies may provide a distorted reflection of the diagnostic performance of RNATs in unselected patients with acute pharyngitis seen in primary care. Second, most studies (35/38, 92%) evaluated RNATs in a laboratory setting, questioning the applicability of the results in a POC clinical setting. Third, because we carried out the analysis at the test evaluation level, three studies were included more than once in the meta-analysis, which may lead to the overrepresentation of a subset of patients. However, these duplicate individuals represent only a small fraction (10%) of the included test results. Fourth, we included ssDNA tests that have lower sensitivity (i.e. 90.5%). This technique without an amplification step has a turnaround time of about 2 hours and has now been replaced by newer molecular methods. Nonetheless, summary estimates were robust in the sensitivity analysis that excluded this technique.

Conclusions

Several RNAT commercial kits for detecting GAS in patients with pharyngitis are available. They are highly accurate and more sensitive than RADTs. On average, RNAT sensitivity and specificity are both above 95%, but whether RNATs can replace RADTs and culture will depend on local epidemiology, clinical needs and available resources. There is enough evidence from laboratory settings, but further studies are needed to conclude regarding their use as POC tests in clinical settings.

Transparency declaration

The authors have stated that there are no conflicts of interest.

Author contributions

JFC and MC conceived and supervised the study. Study selection was by CD and JFC; data extraction was by CD and YR. The first draft of the manuscript was written by CD and all authors contributed to critical revision of the manuscript. All authors approved the final version.

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Ethical approval

Not required.

Previous presentation

Preliminary study results were presented in part at the 8th Congress of the European Academy of Paediatric Societies (Virtual Congress), October 2020.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.04.021.

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