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Review

Nucleic acid amplification techniques for the detection of *Schistosoma mansoni* infection in humans and the intermediate snail host: a structured review and meta-analysis of diagnostic accuracy

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ABSTRACT

Background: Schistosomiasis is a parasitic disease caused by hematodes of genus *Schistosoma*. This review evaluated the available nucleic acid amplification techniques for diagnosing *S. mansoni* infections in humans, intermediate host snails, and presumed rodent reservoirs.
Methods: Sensitivity, specificity, diagnostic odds ratio (DOR), and 95% CI were calculated based on available literature. The potential of PCR, nPCR, PCR-ELISA, qPCR, and LAMP was compared for diagnosing *S. mansoni* infections.
Results: A total of 546 published records were identified. Quality assessment by QUADAS-2 revealed an uncertain risk in most studies, and 21 references were included in the final. For human samples, the four nucleic acid amplification techniques showed an overall sensitivity of 89.79% (95% CI: 83.92%-93.67%), specificity of 87.70% (95% CI: 72.60%-95.05%), and DOR of 37.73 (95% CI: 21.79-65.33). LAMP showed the highest sensitivity, followed by PCR-ELISA, PCR, and qPCR, while this order was almost reversed for specificity; qPCR had the highest AUC. For rodent samples, qPCR showed modest sensitivity (68.75%, 95% CI: 43.32%-86.36%) and high specificity (92.45%, 95% CI: 19.94%-99.83%). For snail samples, PCR and nPCR assays showed high sensitivity of 90.06% (95% CI: 84.39%-93.82%) and specificity of 85.51% (95% CI: 54.39%-96.69%).
Conclusion: Nucleic acid amplification techniques had high diagnostic potential for identifying *S. mansoni* infections in humans.

Introduction

Schistosomiasis is a disease in the definitive mammalian host caused by adult trematode worms producing eggs that release miracidia when excreted with faeces or urine (depending on the species) into freshwater, where they infect the intermediate snail host (LoVerde, 2019). It is an important neglected tropical disease in the endemic tropical and subtropical areas and has been reported from 78 countries (WHO, 2020). Currently, an estimated 236 million people require preventative chemotherapy

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(McManus et al., 2018; WHO, 2020), and more than 90% live in Africa, resulting in an estimated combined loss of 2.5 million disability-adjusted life years (DALYs) (WHO, 2020). There are six schistosome species infective to man: *Schistosoma haematobium* (*S. haematobium*), *S. mansoni, S. intercalatum, S. guineensis, S. japonicum,* and *S. mekongi* (McManus et al., 2020), the first of which causes urogenital schistosomiasis, while infection by any of the other species results in the intestinal form of the disease. The difference depends on the preferable sites of the adult worms in the definitive host, where the intestinal form produces hepatomegaly, ascites, and other liver-related complications because parasite eggs generally end up in this organ. *S. haematobium* produces urological problems and might also cause genital pathologies, with particular impact on female patients since the eggs of this species become trapped in the bladder wall and its surroundings (Hotez et al., 2019; McManus et al., 2020). *S. haematobium* is endemic in the African continent and in pockets of the Arabian peninsula; *S. mansoni* in Africa and Latin America; *S. intercalatum* and *S. guineensis* in limited areas of West Africa; *S. japonicum* in the People's Republic of China and the Philippines, with minor foci in Indonesia; and *S. mekongi* in Cambodia and Lao People's Democratic Republic (McManus et al., 2020).

The global recommended strategy for the control of schistosomiasis is preventive using praziquantel for chemotherapy (WHO, 2002a), but it has become clear that additional complimentary measures will be needed to achieve elimination of the disease (WHO NTDs 2013; Knopp et al., 2019). *S. mansoni* is transmitted by infected, intermediate host snails belonging to the genus *Biomphalaria* (Hailegebriel et al., 2020). While reliable, accurate, and sensitive diagnosis is essential for the control and ultimate goal of schistosomiasis elimination, and is crucial for the ongoing validation of vaccine candidates in clinical trials (Ogongo et al., 2018).

To date, microscopic examination by the Kato-Katz thick faecal smear technique (WHO, 2002b) has been the reference standard for the diagnosis of *S. mansoni* infection in humans. For snail diagnosis, shedding and crushing methods are common examination methods when searching for cercariae, the stage that completes the parasite's life cycle when penetrating human skin (Farghaly et al., 2016). Although these methods are simple, cost-effective, and can detect eggs or cercariae, their sensitivity is low, especially in post-preventive chemotherapy areas characterized by low-prevalence infections (Fuss et al., 2018; Pontes et al., 2003). Employment of immunological techniques, such as tests for circulating schistosome antigens, is another option for the diagnosis of infection (Fuss et al., 2018). A commercially available test targeting the circulating cathodic antigen (CCA), the point-of-care (POC), can be used to detect a current *S. mansoni* infection in urine samples (Colley et al., 2020). However, testing for circulating antigens emanating from the various other species raises the question of specificity, which remains a problem that needs to be solved. An increasing number of molecular techniques (Weerakoon et al., 2018) – including polymerase chain reaction (PCR) (Pontes et al., 2003), different adoptions of this approach (Guegan et al., 2019), and the loop-mediated isothermal amplification (LAMP) (Gandasegui et al., 2018; Hamburger et al., 2013; Qin et al., 2018) are currently being used to detect schistosome infections in various hosts. However, the definitive host is generally only humans, except in chistosomiasis japonica and Schistosomiasis mekongi, which, in contrast to all other species, are zoonotic forms of the infection. *S. mansoni* sometimes also infects non-human primates (Kebede et al., 2020; Richards et al., 2019) and various rodent species (Gentile et al., 2011).

PCR was the first detection system based on amplification of the worm DNA to be widely used for schistosomiasis diagnosis (Abe et al., 2018; Weerakoon et al., 2018). The nested PCR (nPCR) involves two primers in two successive runs of the test, where the second amplifies a secondary target within the product of the first run. The advantage is that this limits non-specificity by only permitting a low number of first runs (Bruscky et al., 2016). Quantitative PCR (qPCR), also named real-time PCR, monitors the amplification of the targeted DNA in real time, as opposed to only measuring the end product, which not only results in higher specificity but also provides a value of the infection intensity (Guegan et al., 2019). While PCR applications belong to the variable-temperature nucleic acid amplification techniques (NAATs), the one-step LAMP, with its two inner primers and two outer primers, amplifies DNA at the same temperature. Other similar techniques (e.g., recombinase polymerase amplification (RPA) (Archer et al., 2020) and recombinase-aided isothermal amplification (RAA) (Zhao et al., 2020)) have also been used for the diagnosis of schistosomiasis but were not included in this search.

Molecular techniques are based on specific gene sequences, and different studies might have used different techniques and sequences, resulting in variability of diagnostic values. However, due to their generally high levels of sensitivity and specificity, these techniques would be useful for identifying infections in low-prevalence areas. Therefore, this review focused on evaluating frequently used NAATs, and *S. mansoni* was selected as being the most widespread species in human schistosomiasis.

Methods

The meta-analysis presented here was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al, 2010), and was registered in the International Prospective Register of Systematic Reviews (PROSPERO no. CRD42021233829) (PROSPERO, 2021). The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (Whiting et al., 2011) was used to assess the reference quality, which was judged by several signalling questions. The risk of bias of each reference was explored according to four criteria: (i) patient selection; (ii) index test; (iii) reference standard; and (iv) assay characteristics, such as flow and timing. Signalling questions were included to help judge the risk of bias, which was judged as "low", "high", or "unclear". If all signalling questions for a criterion could be positively answered, the risk of bias due to this particular criterion was judged as low. The unclear category was only used with reference to articles with insufficient data. In addition, applicability concerns were also assessed in the same way for the criteria covering patient selection, index test, and reference standard.

Search strategy and data collection

The search strategy is available in PROSPERO (PROSPERO, 2021). PubMed, Web of Science, African Journals Online, the Cochrane Library, and the China National Knowledge Infrastructure (CNKI), a key national research and information publishing institution in the People's Republic of China, were searched for diagnosis of *S. mansoni* infections. No limitations were set for language, survey, or reference type. The last search was performed on 16 December 2020. References were selected according to the eligibility criteria and judged by two researchers. In cases where two judgment, a third senior scientist was consulted until agreement was reached. After eliminating duplicates, all references were initially screened based on title and abstract; if needed, the whole article was examined. EndNote version X8 was used to manage the references selected. The quality assessment of references was done using Review Manager version 5.3 (RevMan 5.3) (Cochrane, 2021). The following information was obtained from the reference papers: title, publica-

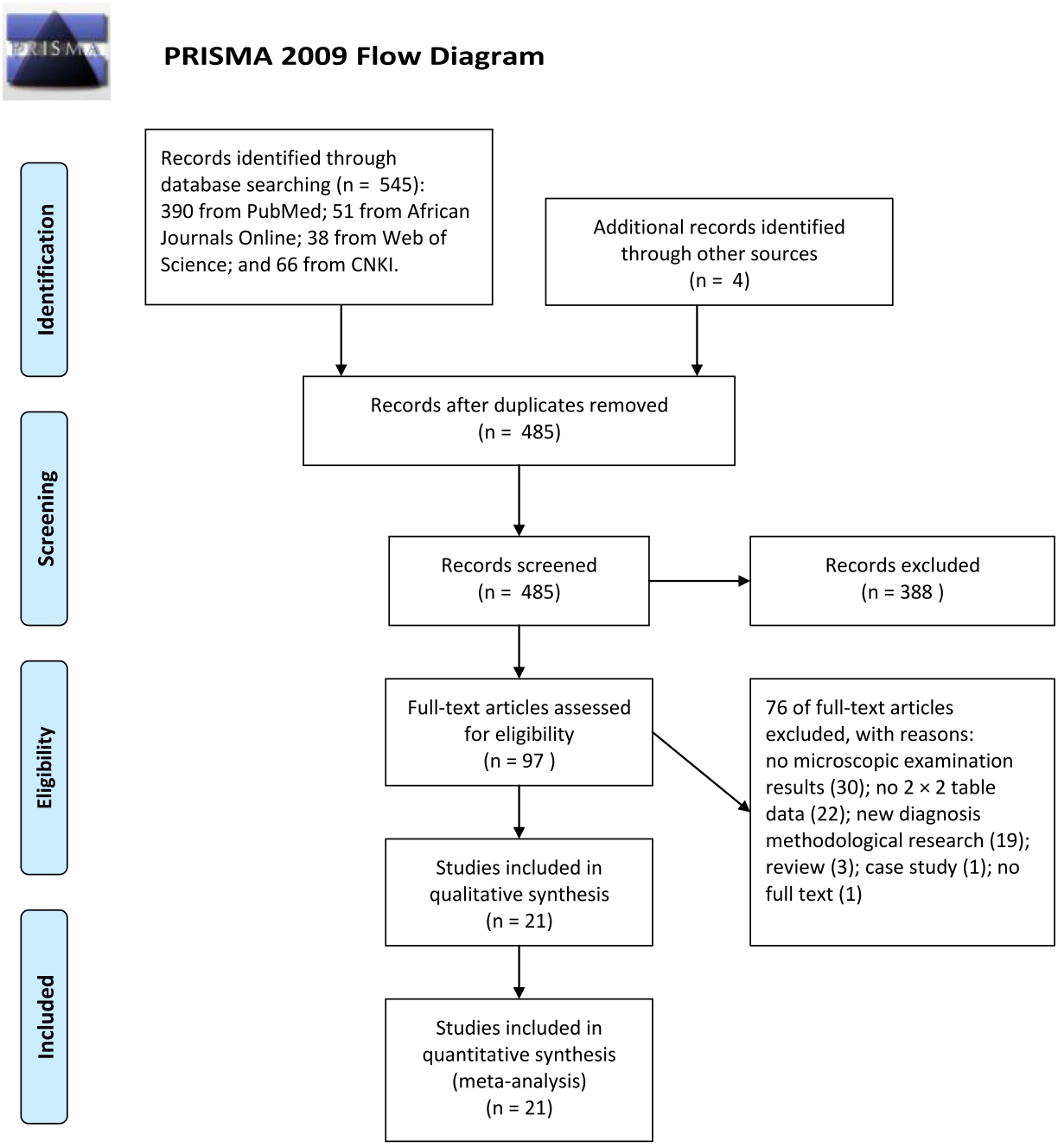

Figure 1. PRISMA flow chart of the article selection.
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement.

tion year, author(s), country, assessment approach, NAATs applied, microscopic examination used, number of samples (N), number of true positives (TP), number of false positives (FP), number of false negatives (FN), and number of true negatives (TN). If one article contained data obtained from different techniques, each set was considered a separate study.

Eligibility criteria

Studies were selected if they included NAAT results and used microscopic examination as reference standard for identification of eggs or cercariae of *S. mansoni*, such as Kato-Katz or formalin ether sedimentation (Sady et al., 2015) for diagnosis in humans, while snail crushing and/or cercarial shedding were required for snail diagnosis. Moreover, the studies should also have included at least one NAAT as the index test (i.e., PCR, nPCR, qPCR, RT-PCR, and LAMP). In addition, the accuracy of the data of both reference standard and index test in each paper should be detailed enough to construct a 2 × 2 table. The 2 × 2 tables were built by crossing the results of reference standard and index test, and contained information to assess the diagnostic accuracy, including TP, FP, FN, and TN. Exclusion criteria focused on absence of 2 × 2 table data or if they had tested less than 10 samples. No papers covering techniques, such as RPA, RAA, DNA sequencing or DNA microarray, were included in this meta-analysis. Studies exclusively dealing with human infections or exclusively dealing with infected snails were excluded, as were short communications, opinion articles, case reports, and reviews.

Table 1
Characteristics of studies extracted from articles included in the meta-analysis.

First author, year	Country	Study design	Test sample	Reference standard	Index test
Fernanda, 2020a (Magalhães et al., 2020)	Brazil	Cross-sectional study	Stool (human)	Kato-Katz slides (n=2)	RT-PCR
Fernanda, 2020b (Magalhães et al., 2020)	Brazil	Cross-sectional study	Stool (human)	Kato-Katz slides (n=6)	RT-PCR
Ebrima, 2020a (Joof et al., 2020)	UK	Laboratory study	Snail (laboratory bred)	Cercarial shedding	PCR
Ebrima, 2020b (Joof et al., 2020)	UK	Laboratory study	Snail (laboratory bred)	Cercarial shedding	PCR
Price, 2019a (Price et al., 2019)	Zambia	Cross-sectional study	Urine (human)	Kato-Katz	PCR
Price, 2019b (Price et al., 2019)	Zambia	Cross-sectional study	Urine (human)	Kato-Katz	PCR
Price, 2019c (Price et al., 2019)	Zambia	Cross-sectional study	Urine (human)	Kato-Katz	LAMP
Price, 2019d (Price et al., 2019)	Zambia	Cross-sectional study	Urine (human)	Kato-Katz	LAMP
Ibrahim, 2018 (Mwangi et al., 2018)	Kenya	Field investigation	Stool (human)	Kato-Katz	LAMP
Gandasegui, 2018a (Gandasegui et al., 2018)	Brazil	Field investigation	Stool (human)	Kato-Katz	LAMP
Gandasegui, 2018b (Gandasegui et al., 2018)	Brazil	Field investigation	Stool (human)	Kato-Katz	LAMP
Gandasegui, 2018c (Gandasegui et al., 2018)	Brazil	Field investigation	Stool (human)	Kato-Katz	LAMP
Adel, 2016a (Farghaly et al., 2016)	Egypt	Field investigation	Snail (field)	Cercarial shedding (autumn)	PCR
Adel, 2016b (Farghaly et al., 2016)	Egypt	Field investigation	Snail (field)	Cercarial shedding (spring)	PCR
Adel, 2016c (Farghaly et al., 2016)	Egypt	Field investigation	Snail (field)	Snail crushing (autumn)	PCR
Adel, 2016d (Farghaly et al., 2016)	Egypt	Field investigation	Snail (field)	Snail crushing (spring)	PCR
Siqueira, 2015a (Siqueira et al., 2015)	Brazil	Field investigation	Stool (human)	Kato Katz-slides (n=2)	PCR-ELISA
Siqueira, 2015b (Siqueira et al., 2015)	Brazil	Field investigation	Stool (human)	Kato Katz-slides (n=12)	PCR-ELISA
Senra, 2018a (Senra et al., 2018)	Brazil	Field investigation	Stool (human)	Kato-Katz	PCR-ELISA
Senra, 2018b (Senra et al., 2018)	Brazil	Field investigation	Stool (human)	Kato-Katz	PCR-ELISA
Schunk, 2015a (Schunk et al., 2015)	Ethiopia	Community survey	Stool (human)	Kato-Katz	RT-PCR
Schunk, 2015b (Schunk et al., 2015)	Ethiopia	Community survey	Stool (human)	Kato-Katz	RT-PCR
Sady, 2015a (Sady et al., 2015)	Yemen	Cross-sectional Study	Stool (human)	Kato-Katz [a]	PCR
Sady, 2015b (Sady et al., 2015)	Yemen	Cross-sectional Study	Stool (human)	Kato-Katz [a]	PCR
Sady, 2015c (Sady et al., 2015)	Yemen	Cross-sectional Study	Stool (human)	Kato-Katz [a]	RT-PCR
Meurs, 2015a (Meurs et al., 2015)	Senegal	Community survey	Stool (human)	Kato-Katz	PCR
Meurs, 2015b (Meurs et al., 2015)	Senegal	Community survey	Stool (human)	Kato-Katz slides (n=2)	PCR
Meurs, 2015c (Meurs et al., 2015)	Kenya	Field investigation	Stool (human)	Kato-Katz	PCR
Meurs, 2015d (Meurs et al., 2015)	Kenya	Field investigation	Stool (human)	Kato-Katz slides (n=2)	PCR
Meurs, 2015e (Meurs et al., 2015)	Kenya	Field investigation	Stool (human)	Kato Katz slides (n=3)	PCR
Espírito-Santo, 2014a (Espírito-Santo et al., 2014)	Brazil	Cross-sectional study	Stool (human)	Kato-Katz	qPCR
Espírito-Santo, 2014b (Espírito-Santo et al., 2014)	Brazil	Cross-sectional study	Stool (human)[b] Serum (human)[b]	Kato-Katz qPCR	qPCR
Carneiro, 2013 (Carneiro et al., 2013)	Brazil	Field investigation	Stool (human)	Kato-Katz	PCR
Enk, 2012 (Enk et al., 2012)	Brazil	Field investigation	Stool (human)[c] Urine (human)[c]	Kato Katz PCR	PCR
Carvalho, 2012 (Carvalho et al., 2012)	Brazil	Field investigation	Stool (human)	Kato-Katz slides(n=2)	PCR
Gentile, 2011a (Gentile et al., 2011)	Brazil	Laboratory evaluation	Stool (water rodent[d])	Kato-Katz	qPCR
Gentile, 2011b (Gentile et al., 2011)	Brazil	Laboratory evaluation	Stool (water rodent[d])	Kato-Katz	qPCR
Oliveira, 2010a (Oliveira et al., 2010)	Brazil	Field investigation	Stool (human)	Kato-Katz	PCR
Oliveira, 2010b (Oliveira et al., 2010)	Brazil	Field investigation	Stool (human)	Kato-Katz	PCR
Gomes, 2009 (Gomes et al., 2009)	Brazil	Field investigation	Stool (human)	Kato-Katz	PCR
Allam, 2009 (Allam et al., 2009)	Egypt	Field investigation	Stool (human)	Kato-Katz	PCR
Melo, 2006a (Melo et al., 2006)	Brazil	Field investigation	Snail (field)	Cercarial shedding	PCR
Melo, 2006b (Melo et al., 2006)	Brazil	Field investigation	Snail (field)	Cercarial shedding	nPCR
Melo, 2006c (Melo et al., 2006)	Brazil	Field investigation	Snail (field)	Cercarial shedding	nPCR
Pontes, 2003a (Pontes et al., 2003)	Brazil	Field investigation	Stool (human)	Kato-Katz slides (n=3)	PCR
Pontes, 2003b (Pontes et al., 2003)	Brazil	Field investigation	Stool (human)	Kato-Katz slides (n=3)	PCR

[a] and formalin ether sedimentation
[b] stool was used for Kato-Katz, while serum for qPCR
[c] stool was used for Kato-Katz, while urine for PCR
[d] *Nectomys squamipes*

Statistical analysis

The data collected were analysed using the R Foundation for Statistical Computing Platform (R software, version 4.0.4). Since the package "mada", a tool for meta-analysis of diagnostic accuracy in R software, only provides statistic sensitivity, specificity, and diagnostic odds ratio (DOR) of the individual studies, the "meta" package was used to present the summary statis-

Table 2
Summary of selected articles by source and technique.

Source	Type of NAAT*	Articles/ studies/ samples (no.)	Subtotal articles/ studies/ samples by source (no.)	Total articles/ studies/samples (no.)
Human	PCR	10 / 18 / 4,704	19 / 35 / 8,381	21 / 46 / 9,533
	PCR-ELISA	2 / 4 / 814		
	qPCR	4 / 7 / 2,070		
	LAMP	3 / 6 / 793		
Snail	PCR	3 / 7 / 1,082	3 / 9 / 1,114	
	nPCR	1 / 2 / 32		
Animal	qPCR	1 / 2 / 38	1 / 2 / 38	

* NAATs: Nucleic acid amplifying technique

tics (Schwarzer, 2021), while “mada” was reserved for assessment of the summarized receiver-operating characteristic (sROC) curves (Doebler and Holling, 2020, Schwarzer, 2021). To present the contributed heterogeneity of individual studies (Shim et al., 2019), the univariate random effect model of the "meta" package (Schwarzer, 2021, Schwarzer et al., 2015, Shim et al., 2019) was used to calculate sensitivity, specificity, and DOR with 95% confidence interval (CI). Forest plots (Schwarzer, 2021) were performed to visualize the corresponding statistics for each study and pooled effects, while tests such as Cochrane’s Q (Higgins et al., 2021) and I^2 according to Higgins (Higgins et al., 2021) were applied to assess study heterogeneity (Higgins et al., 2021). Differences at $p<0.05$ were considered as statistically significant for the Cochrane’s Q test, while the Higgins’ I^2 presented the degree of heterogeneity as 0-40% = little importance; 30-60% = moderate; 50-90% = substantial; and 75-100% = high. All studies were divided into different sample sources to show the corresponding statistical results. A correction value of 0.5 was entered if "0" appeared in the data cell of a study.

The performance of the diagnostic tests was assessed according to the sROC curve (Walter, 2002) along with the pooled area under the curve (AUC) for each type of technique, using a bivariate approach (the Reitsma function in the "mada" package) (Doebler and Holling, 2020). The closer the sROC curve was to the upper left, the more reliable the test, while the higher the AUC value, the better the test performance. Moreover, to explore the relationship between the various techniques and heterogeneity, and elucidate sensitivity and false positive results comparing two techniques, bivariate meta-regression analysis available in the "mada" package (Doebler and Holling, 2020) was used. Only heterogeneity values at $p<0.05$ of the likelihood-ratio test were accepted.

Results

Study selection

In total, 545 records were identified from the search of the electronic databases, from which 64 articles were removed due to duplication and 388 excluded based on non-relevant titles and/or abstracts, while 97 were subjected to further review, after which 76 further articles were excluded. The overall selection progress is shown in Figure 1. Taken together, 21 articles met for inclusion criteria. Table 1 shows data extracted from the 21 articles on research carried out in different countries using different diagnostic assays. The samples collected varied and consisted of serum, stool, and urine samples from humans, water rodent stools, and whole snails. In most articles, more than one single NAAT was used for the diagnosis and several articles used different reference methods. Hence, reference standards and index tests are also listed. The obtained data were classified into three groups based on the source of the test samples: (i) human; (ii) rodent; and (iii) snail. As seen in Table 2, five techniques were included in this analysis. Table 2 further shows the summary of the number of articles, including the studies and samples referred to in the article in question. Overall, 46 studies and 9,533 test samples were collected from the 21 articles.

Quality assessment and risk of bias

As seen in Figure 2, there was an unclear risk of bias according to the QUADAS-2 assessment. It originated in three areas: (i) in the patient selection (16/21 or 76.2%), where consecutive or random sampling had not been properly applied during enrolment; (ii) in the index test (19/21 or 90.5%), where the NAAT results had been interpreted with knowledge of the results of the reference standard; and (iii) with respect to flow and timing of the assay (7/21 or 33.3%), where all enrolled patients had not been included. A few of these unclear risks of bias were related to the reference standard. In addition, it was also observed that "high risk" of applicability was detected in the patient selection and index test.

Diagnostic accuracy of nucleic acid amplification tests in humans

In total, 35 studies and 8,381 human test samples were subjected for this analysis. Four NAATs (PCR, PCR-ELISA, qPCR, and LAMP) were used in diagnosing the human test samples. The sensitivity, specificity, and DOR, including 95% CI and heterogeneity, related to these techniques are presented in Figures 3-5. As seen in Figure 3, the overall heterogeneity of sensitivity was 89.79% (95% CI: 83.92%-93.67%), with a Higgins’ I^2 outcome in sensitivity analysis of 87%, and Cochrane Q at $p<0.01$. Figure 4 shows that the overall specificity was 87.70% (95% CI: 72.60%-95.05%), with high heterogeneity among different human source studies (I^2 = 95% and Cochrane Q at $p<0.01$). Figure 5 shows an overall DOR of 37.73 (95% CI: 21.79-65.33), with high heterogeneity across all studies (I^2 = 80% and Cochrane Q at $p<0.01$).

Comparison of sROC curves in human test samples

Following the bivariate analysis model using the "reitsma" function in mada, sROC curves comparing the diagnostic performance of the different NAATs in the human test samples were obtained (Figure 6). Although the sROC plots slightly overlapped and the confidence regions significantly so, it was concluded from the AUC values that qPCR is the most reliable technique in diagnosing human samples, followed by PCR-ELISA, LAMP, and PCR, in that order. Through the bivariate meta-regression analysis, the combined effect of sensitivity and FPs in each two techniques were compared with a likelihood-ratio test. No differences were identified for PCR vs PCR-ELISA (p=0.26), PCR vs LAMP (p=0.08), or PCR-ELISA vs LAMP (p=0.36); however, differences were detected for PCR vs qPCR ($p<0.01$), PCR-ELISA vs qPCR ($p<0.01$), and qPCR vs LAMP ($p<0.01$). The overall outcome of these comparisons was that qPCR performed better than any of the other diagnostic assays.

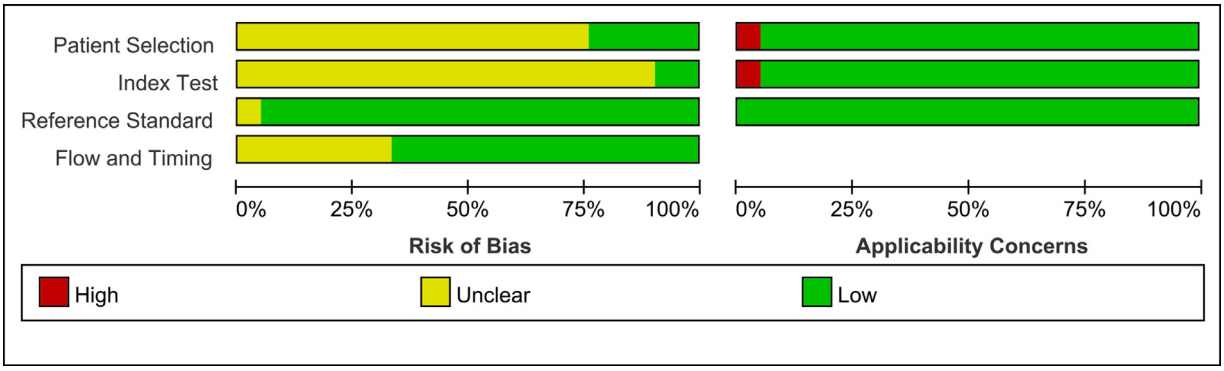

Figure 2. Quality assessment of 21 articles using QUADAS-2.
Proportion represents the summary for risk of bias and applicability
QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies

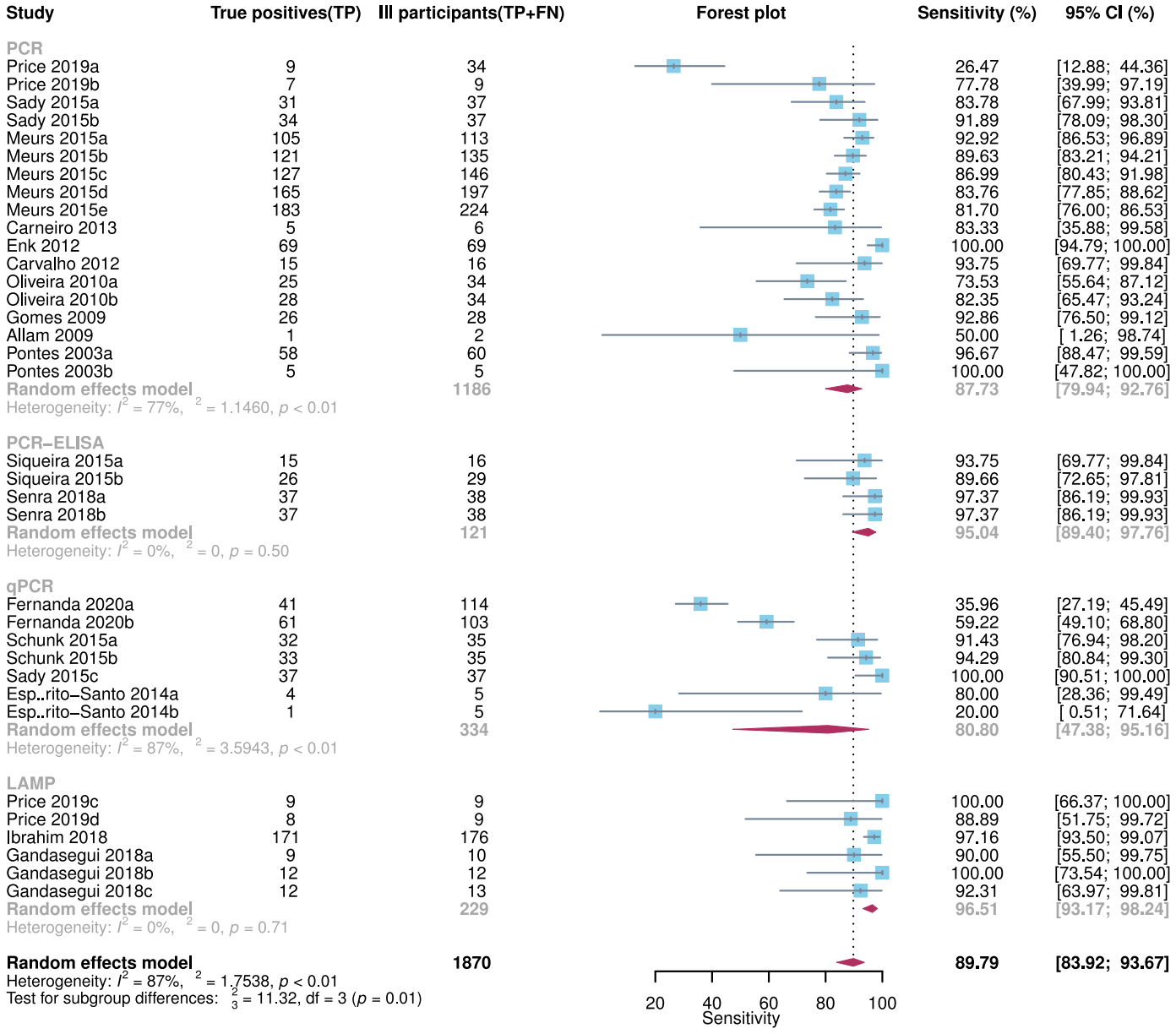

Figure 3. Human studies: forest plots for sensitivity determination.
The studies are shown by different NAATs
The sensitivity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates

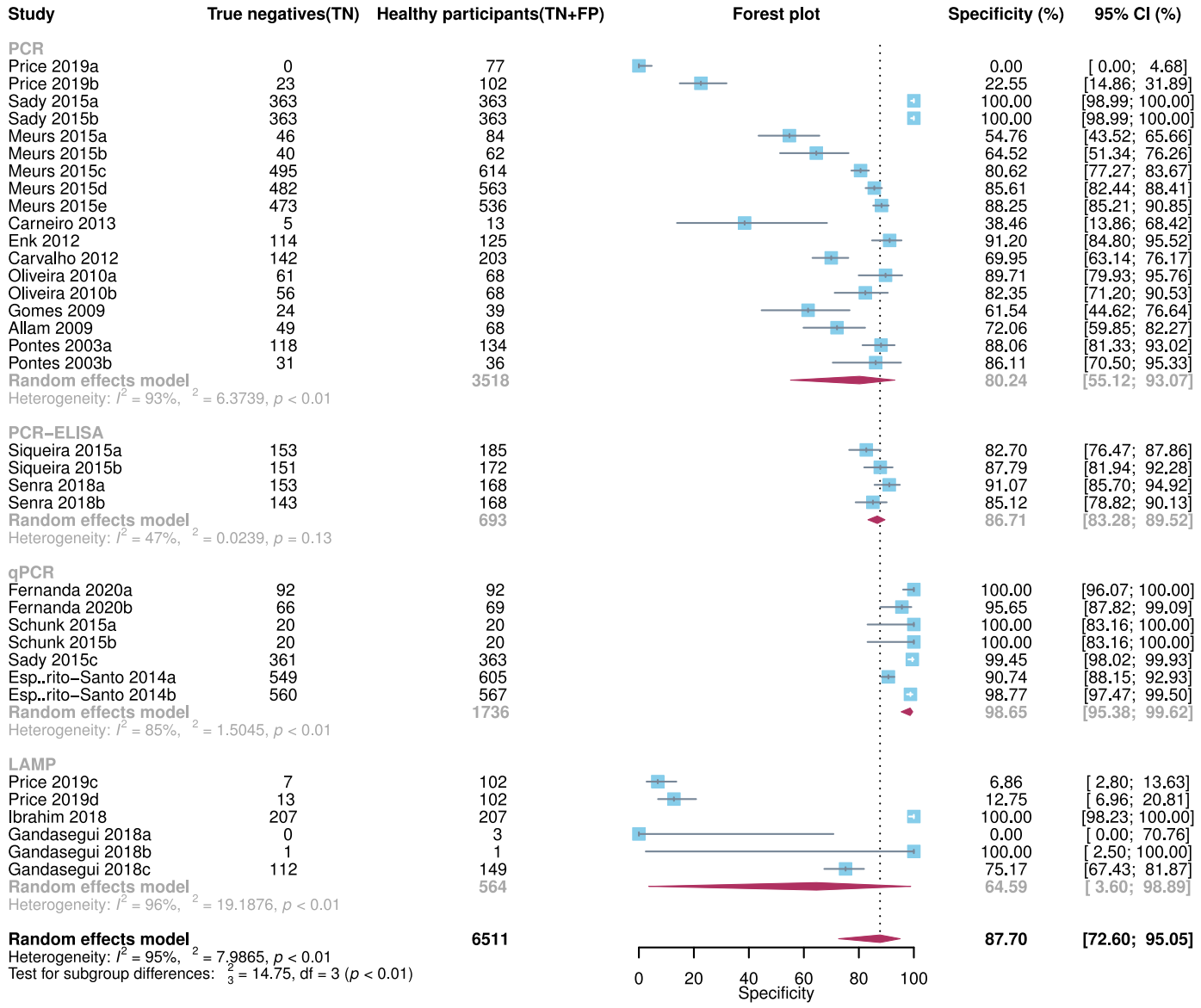

Figure 4. Human studies: forest plots for specificity determination.
The studies are shown by different NAATs
The specificity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates

These results also imply that the heterogeneity of the results of these primary studies might be explained by the use of different NAATs.

Diagnostic accuracy of nucleic acid amplification assays in snails

This analysis included nine snail studies describing a total of 1,114 snail samples subjected to testing, and two NAATs (i.e., PCR and nPCR). The summary of sensitivity, specificity, and DOR in each study is shown in Figures 7-9.

Comparison of sROC curves for the snail samples

As can be seen from the sROC curves of the two NAATs in detecting snail samples (Figure 10), the summary estimates and the confidence regions were separated. A significant difference was found when PCR was compared with nPCR through bivariate meta-regression analysis (p<0.01). It follows that PCR is a more reliable test for snail diagnosis than nPCR.

Diagnostic accuracy of nucleic acid amplification assays in animals

Although schistosomiasis mansoni is principally a human disease, it has been detected uncommon in various non-human primates (Richards et al., 2019) nor in rodents (Amaral et al., 2016). Two studies, both using qPCR, to test a total of 38 stool samples from *Nectomys squamipes*, a type of water rodent (Figure 11 A-C), were included in the current analysis.

Discussion

To realize the goal of eliminating schistosomiasis as a public health problem by 2030, as stipulated by the World Health Organization (WHO) (WHO, 2020), the use of accurate diagnostic tests are of considerable importance. For this reason, this study fo-

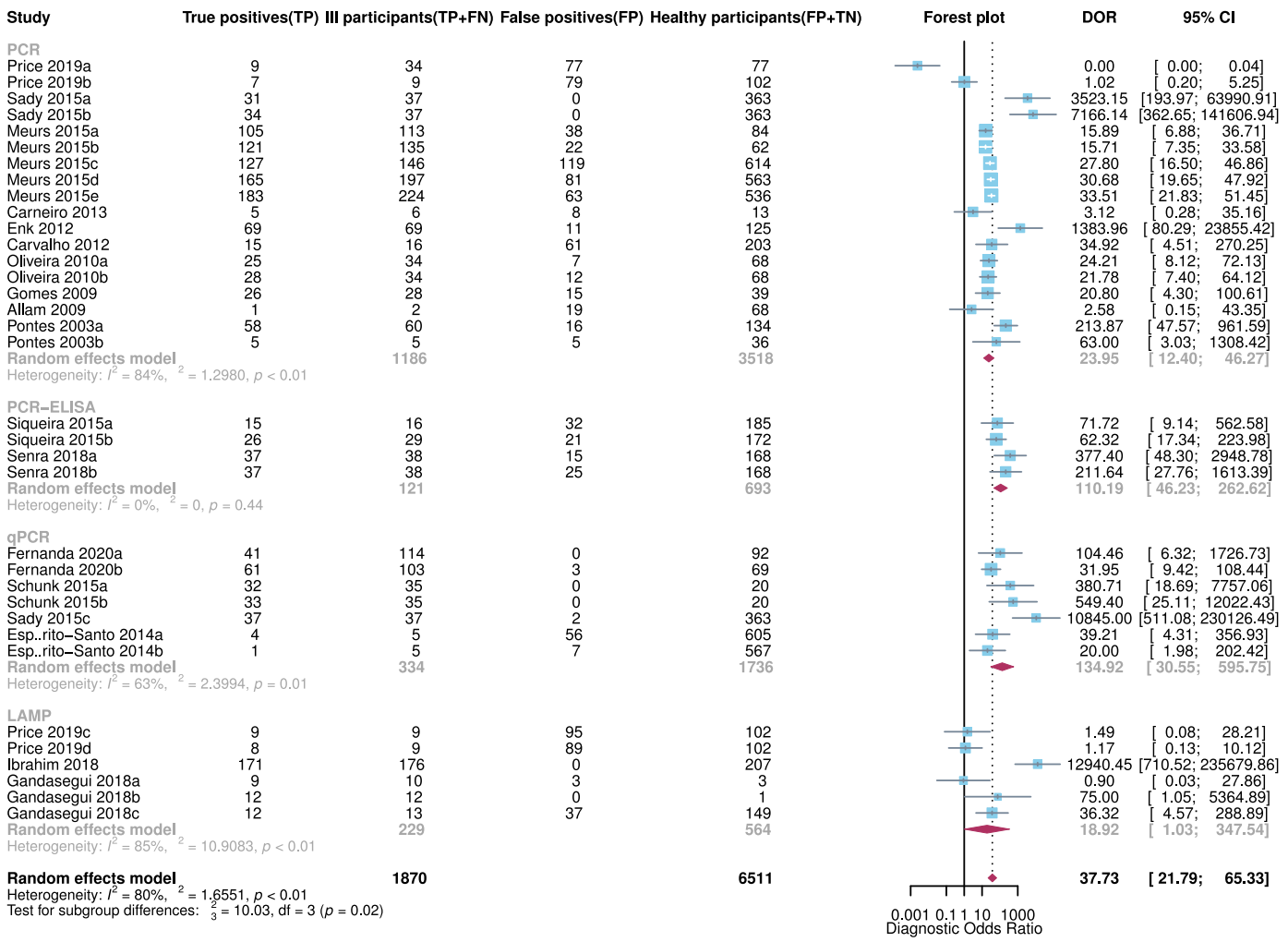

Figure 5. Human studies: forest plots for diagnostic odds ratio determination. The studies are shown by different NAATs The diagnostic odds ratio and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates

cused on the description of NAATS, which have very high sensitivity and specificity. This systematic review is the first meta-analysis of the diagnostic performance of NAATs for *S. mansoni* infections. Although minor variations were found between the investigated techniques, they all performed well for detecting schistosome DNA in human and snail samples, thus supporting the prerequisite of accurate and sensitive diagnostic tests. Compared with widely used microscopic methods, the sensitivity of the examined assays must be deemed excellent.

LAMP has been popular in the diagnosis of various diseases since its discovery in 2000 (Notomi et al., 2000). Although PCR-ELISA, combining the strengths of PCR and ELISA, has demonstrated high sensitivity and previously been useful in confirming suspicious cases (Senra et al., 2018; Siqueira et al., 2015), it should be pointed out that LAMP, in contrast to PCR-based techniques which are expensive and time-consuming, is a user-friendly technique that does not require complex instruments and can, therefore, be operated in the field (Nzelu et al., 2019).

Specificity is defined as the ability to identify TN cases. Low specificity means presence of a high number of FPs, i.e., more cases that are negative will be diagnosed as positive. The qPCR technique showed the highest specificity with respect to human samples. Although its costs are higher than those of conventional PCR, it is highly useful in identifying samples with low parasite burdens when access to specific equipment and software is granted (Weerakoon et al., 2018). In contrast, the specificity of the other four techniques was somewhat disappointing, particularly nPCR, which had the lowest specificity when used for detecting schistosome DNA in snail samples.

The results of the quality assessment using the QUADAS-2 tool showed uncertain risk of bias in most studies, particularly in terms of index test, patient selection, and flow and timing of testing samples. Unlike the design of randomized controlled trials, patients were not consecutively or randomly selected for schistosomiasis diagnosis. Moreover, most studies did not provide details of the use of blinding when performing NAATs. In addition, due to difficulties in the collection of field samples, some of these samples might have been lost in the final analysis.

The sROC curve is a comprehensive indicator of the diagnostic value, considering both sensitivity and specificity. Although the overlap of the summary sROC plots may have affected the visual estimate of the performance of the different techniques, the merged AUC values for qPCR, PCR-ELISA, and LAMP indicated high diagnostic values worthy of further follow-up.

According to the results of the random-effects model, heterogeneity was evident for sensitivity, specificity, and DOR among the investigated studies. This is an important variable that was identified in this study in the form of differences with respect to reference index, sample source, and DNA extraction methods used, as well as variations in study areas and researchers. Regarding the

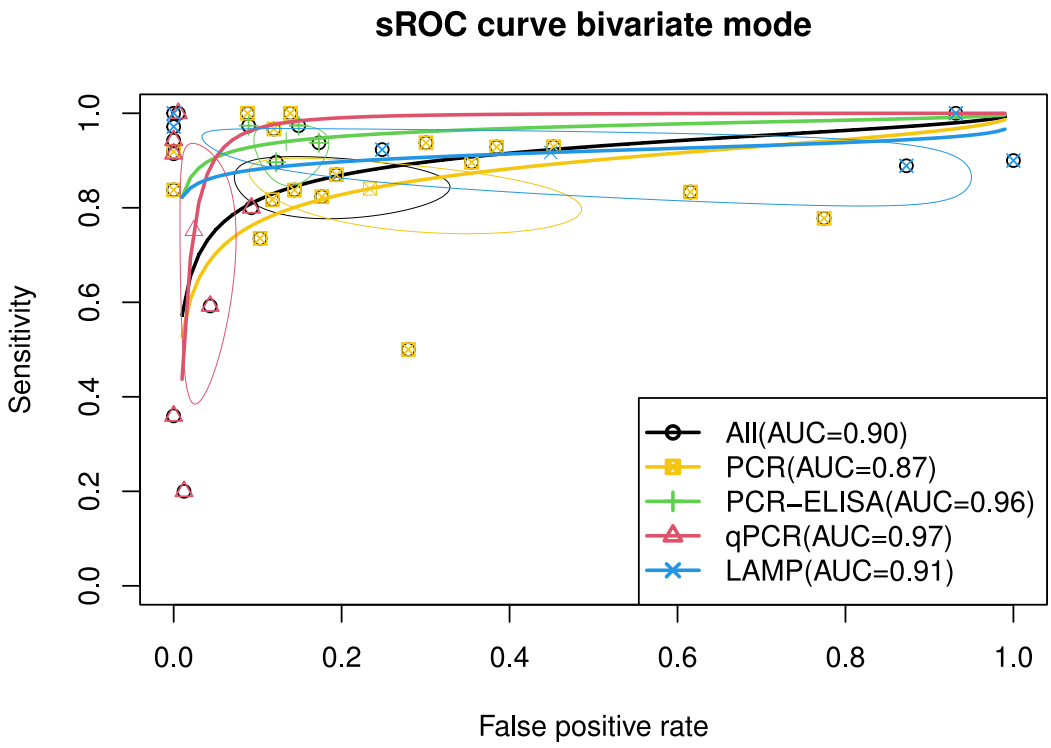

Figure 6. sROC curves and AUC for different NAATs applied for human test samples.
Coloured curves are the sROC curves of different NAATs. Coloured dots indicate individual studies. The areas marked as continuous coloured lines represent the confidence region for the point estimate of the pair of sensitivity and false positive rate.

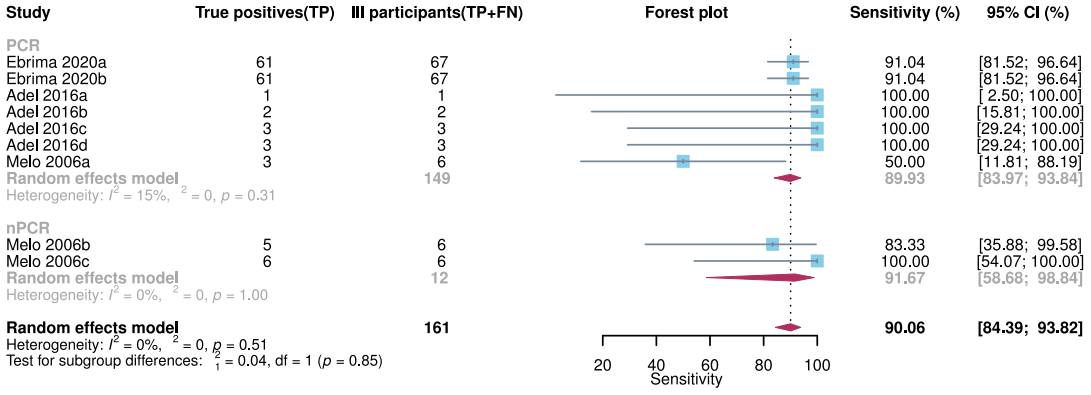

Figure 7. Snail studies: forest plots for sensitivity determination.
The studies are shown by different NAATs
The sensitivity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.

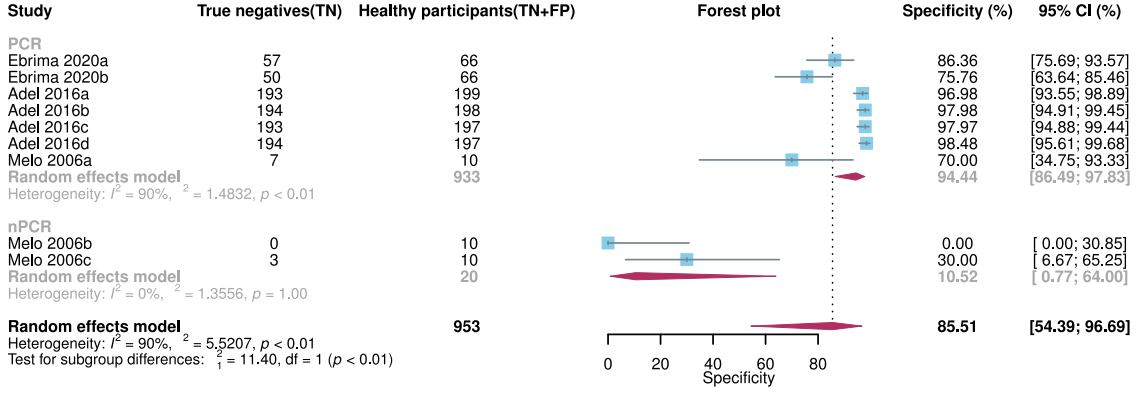

Figure 8. Snail studies: forest plots for specificity determination.
The studies are shown by different NAATs
The specificity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates

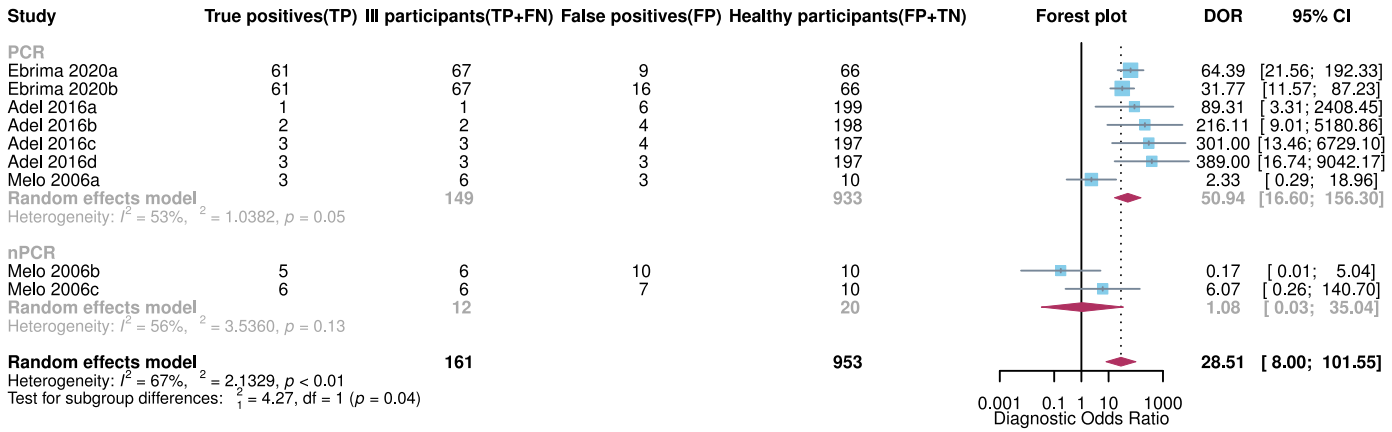

Figure 9. Snail studies: forest plots for diagnostic odds ratio determination.
The studies are shown by different NAATs
The diagnostic odds ratio and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates

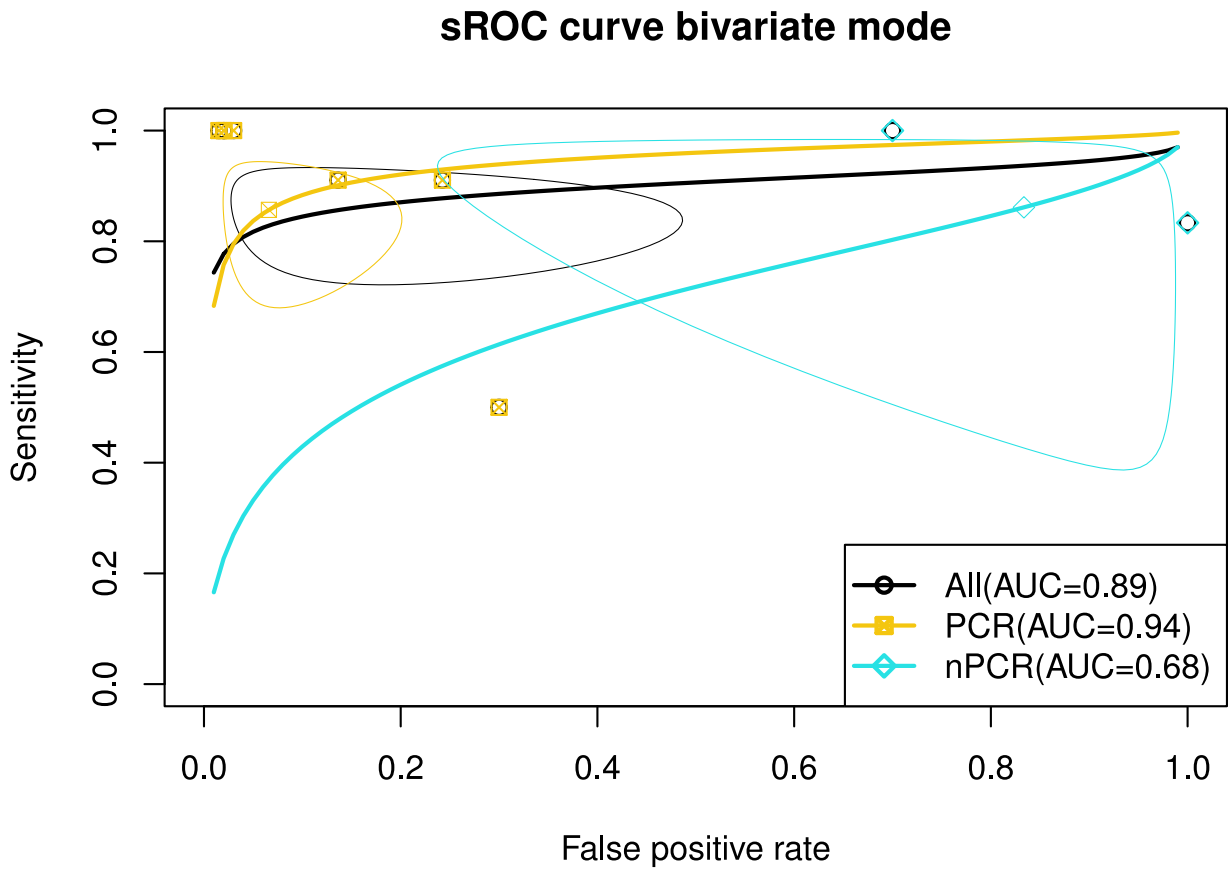

Figure 10. sROC curves of different NAATs in detecting snail samples.
Coloured curves are the sROC curves of different NAATs. Coloured dots indicate individual studies. The areas marked as continuous coloured lines represent the confidence region for the point estimate of the pair of sensitivity and false positive rate.

reference index, it was noticed that Kato-Katz and formalin ether sedimentation (or just ether sedimentation) was the preferential microscopic detection method. There were also considerable differences in how the Kato-Katz thick smear test was carried out (e.g., one faecal sample using one slide; one faecal sample using two slides; three faecal samples using three slides; two slides prepared from each of three faecal samples; or one faecal sample using 12 slides). It was also noticed that different samples (stool, urine, serum, and snail) were approached differently or that different kinds of samples were used in the same study (e.g., microscopy of stool samples was compared with serum testing by a NAAT). It was reported that positivity rates showed significant differences when the same NAAT was utilized to detect both stool samples and serum samples (Espírito-Santo et al., 2014). The different kinds of samples, especially in the same study, might be the main source of heterogeneity of the diagnosis value.

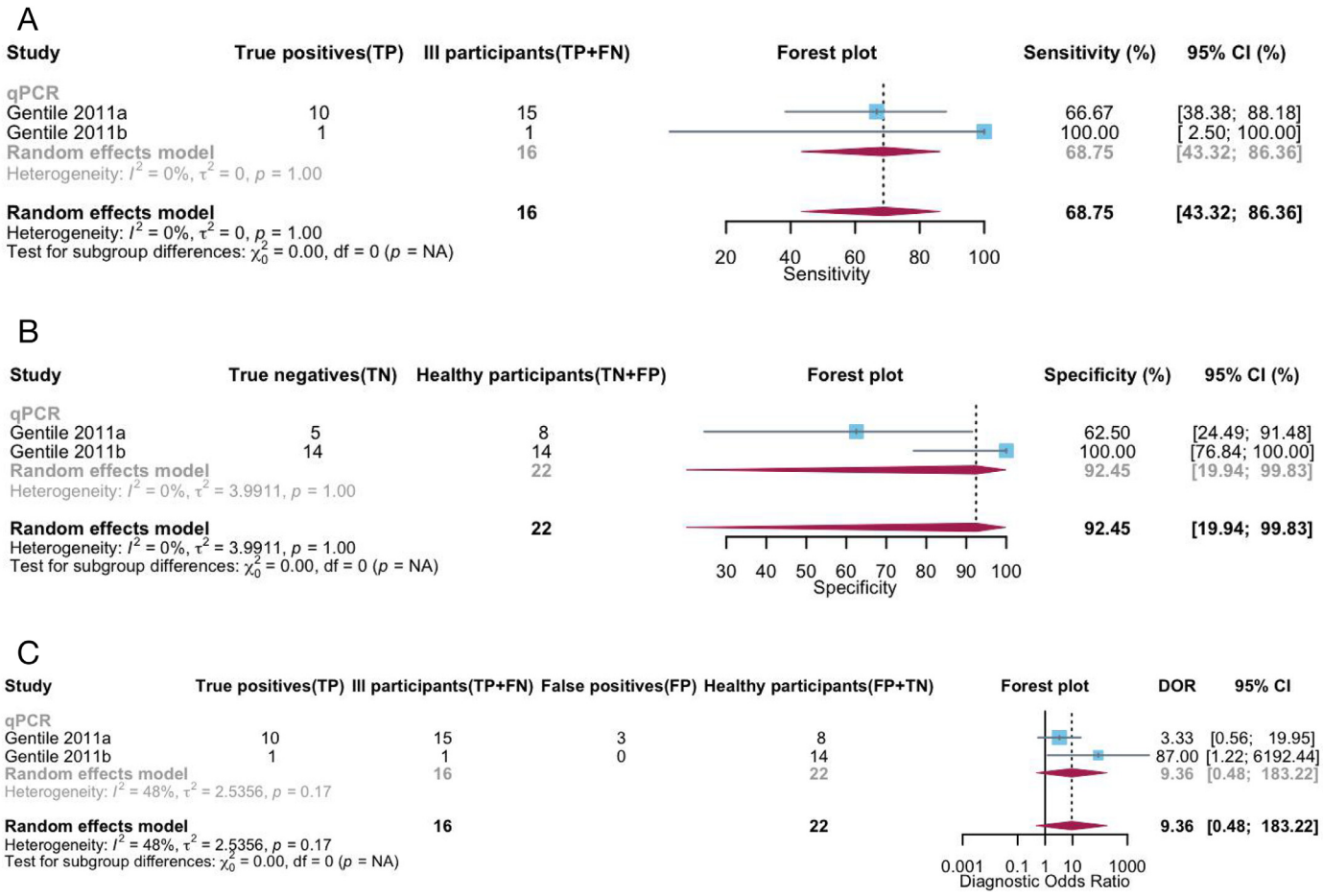

Figure 11. Animal studies: forest plots for sensitivity (A), specificity (B), and diagnostic odds ratio (C) determination.
The sensitivity, specificity, diagnostic odds ratio, and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates

This review had some limitations. First, considering that each study had an important role, no strict criteria were set, which might have added to the overall observed complexity and heterogeneity. The limited number of available articles may have affected the estimated results. Second, for a strict estimate, comparisons should depend on the sample materials that were used and on the stage of infection. However, because of the diversity of technologies and the complex life cycle of schistosomiasis, the effect of different target sequences on the diagnostic reliability of NAAT, as well as the different stages of the schistosoma life cycle, were not considered. Third, because of their free use and open sharing, R packages were used to analyse the data in this review, but no open-access package was available to drive funnel plots, which are used to assess potential publishing bias, in this diagnostic test accuracy. If a publication bias did exist, its true effect may have been underestimated.

Conclusions

The NAATs had a high diagnostic value for identifying *S. mansoni* infections, showing very high sensitivity and specificity. Out of the five approaches, qPCR, PCR-ELISA, and LAMP stood out. Although PCR-ELISA had a higher AUC value, the complexity of sample processing and time consumption limit its usage. LAMP and qPCR are deemed particularly suitable, and hence, warrant further research in view of their potential in large-scale diagnostic surveys. However, the specificity of LAMP needs to be improved.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data sharing

Please contact author for data requests.

Author contributions

H-ML, Z-QQ, RB, M-BQ, SX, SL, NX, JU, and X-NZ initiated and conceptualized the project. H-ML, Z-QQ, and M-BQ pursued the systematic review and collected the data. H-ML, Z-QQ, M-BQ, SX, SL, and X-NZ analysed the data. H-ML, Z-QQ, M-BQ, SX, SL, and X-NZ wrote the original draft, and RB and JU critically edited and revised the manuscript. All authors approved the final version.

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Ethical approval statement

Not applicable.

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