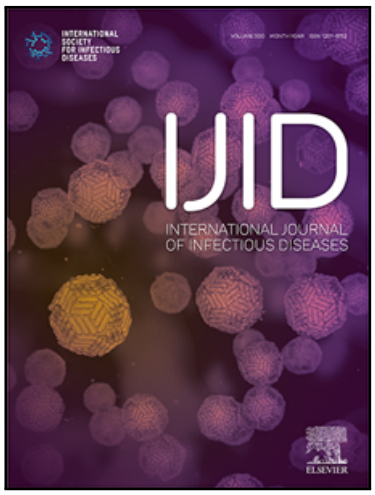

Nucleic acid amplification techniques for the detection of Schistosoma mansoni infection in humans and the intermediate snail host: a structured review and meta-analysis of diagnostic accuracy

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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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Highlights

Nucleic acid amplification techniques for diagnosis of *S. mansoni* infections evaluated

Pooled sensitivity was 0.898 and specificity was 0.877 for human samples

qPCR showed modest sensitivity (0.687) and high specificity (0.925) for rodents

PCR and nPCR assays showed sensitivity of 0.901 and specificity of 0.855 for snails

Summary

Background

Schistosomiasis mansoni, a parasitic disease caused by the most common schistosome species, *Schistosoma mansoni*. This review evaluates available nucleic acid amplification techniques for the diagnosis of *S. mansoni* infections in humans, snails, and presumed rodent reservoirs.

Methods

Sensitivity, specificity, DOR, and 95%-CI were calculated based on available literature. We compared the potential of PCR, nPCR, PCR-ELISA, qPCR, and LAMP for the diagnosis of *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 finally accepted.

For human samples, the four nucleic acid amplification techniques showed an overall sensitivity of 0.898 (95%-CI: 0.839-0.937), a specificity of 0.877 (95%-CI: 0.726-0.950), and a DOR of 37.728 (95%-CI: 21.786-65.335). LAMP showed the best sensitivity, followed by PCR-ELISA, PCR, and qPCR, while this order almost reversed for specificity. qPCR had the highest AUC. For rodent samples, qPCR showed a modest sensitivity (0.687, 95%-CI: 0.433-0.864) and high specificity (0.925, 95%-CI: 0.199-0.998). For snail samples, PCR and nPCR assays showed a high sensitivity of 0.901 (95%-CI: 0.844-0.938) and a specificity of 0.855 (95%-CI: 0.544-0.967).

Conclusion

Nucleic acid amplification techniques have high diagnostic potential for identifying *S. mansoni* infections in humans.

Keywords:

Schistosomiasis; *Schistosoma mansoni*; human diagnosis; snail diagnosis; nucleic acid amplification technique; PCR-ELISA; qPCR; LAMP; PCR; nPCR

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 water where they infect the intermediate snail host that acts as one of the reservoirs of this trematode parasite (LoVerde, 2019). It is an important neglected tropical disease (NTD) in the endemic tropical and subtropical areas and has been reported from 78 countries (WHO, 2020). Currently, an estimated number of about 236 million people require prevention chemotherapy (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. 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. haemat*roduces urologic problems and often also to genital pathologies with particular impact in 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*s endemic on the African continent and in pockets in the Arabian peninsula; *S. mansoni* in Sub-Saharan Africa and Latin America; *S. intercalatum* and *S. guineensis* in limited areas of West Africa; *S. japonicum* in China and the Philippines with minor foci in Indonesia; and *S. mekongi* in Cambodia and Laos (McManus et al., 2020).

Preventive chemotherapy using praziquantel is the global recommended strategy for the control of schistosomiasis (WHO, 2002a), but it has become clear that additional complimentary measures will be needed to achieve elimination of the disease (WHO NTDs, 2013). *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 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 parasite 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 cathodic schistosome antigen (CCA), termed the point of care CCA assay (POC-CCA), 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 the 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 *schistosomiasis japonica* and *schistosomiasis mekongi* that, in contrast to all other species, are zoonotic forms of the infection. *S. mansoni* infects sometimes also non-human primates (Kebede et al., 2020) 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 (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 better 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. Others 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 applied 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 a variability of diagnostic values. However, due their generally high levels of sensitivity and specificity, these techniques would be useful for identifying infections in low-prevalence areas and this review, therefore, focused on evaluating frequently used NAATs. *S. mansoni* was selected for being the most common species in human schistosomiasis.

Methods

The meta-analysis presented here was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) guidelines (Moher D, 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. We explored the risk of bias of each reference according to four criteria: patient selection, index test, reference standard, and assay characteristics, such as flow and timing. Signalling questions were included to help judging the risk

of bias, which was judged as “low”, “high”, or “unclear”. If all signalling questions for a criterion could be answered positively then the risk of bias due to this particular criterion was judged as low. The unclear category was used only 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). We searched 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 China 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 case of controversy, a third senior scientist was included in the selection. After eliminating duplicates, all references were initially screened based on title and abstract; if needed the whole article was viewed. EndNote X8 was used to manage the references selected. The quality assessment of references was presented using Review Manager 5.3 (RevMan 5.3) (Cochrane, 2021)

The following information was obtained from the reference papers: title, publication 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 several different 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 include at least one NAAT as 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 table was built by crossing the results of reference standard and index test and contains 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 also short communications, opinion articles, case reports and reviews.

Statistical analyses

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, we used the “meta” package to present the summary statistics (Schwarzer, 2021), while “mada” was reserved for the 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 show 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 accepted 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 is 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 different technique, we used bivariate meta-regression analysis available in the "mada" package (Doebler and Holling, 2020). Only heterogeneity values at $p<0.05$ of the likelihood-ratio test were accepted.

Results

Study selection

We identified 545 records from the search of the electronic databases mentioned, 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 was showed in Figure 1. We, thus, finally ended up with 21 articles that fitted the inclusion and exclusion criteria.

< Figure 1 about here>

Table 1 shows data extracted from the 21 articles on research carried out in different designs and in different countries. 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. Therefore, reference standards and index tests are also listed.

We classified the obtained data into three groups based on the source of the test samples: human, rodent and snail. As seen in Table 2, five techniques were included in this analysis. The Table further shows the summary of the number of articles including the studies and samples referred to in the article in question. Forty-six studies and 9,533 test samples in all were collected from the 21 articles focused on.

Quality assessment and bias risk

As seen in Figure 2, there was an unclear risk of bias according to the QUADAS-2 assessment. It originated in three areas: 1) in the patient selection (16/21 or 76.2%), where consecutive or random sampling had not been properly applied during enrolment; 2) 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 3) 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 were related to the reference standard. In addition, we observed that "high risk" of applicability was also detected in the patient selection and index test.

< Figure 2 about here>

Diagnostic accuracy of nucleic acid amplification tests in humans

In total, 35 studies and 8, 381 human test samples were collected in 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 0.898 (95% CI: 0.839-0.937) 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 0.877 (95 % CI: 0.726-0.950), 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 37.728 (95 % CI: 21.786-65.335), with high heterogeneity across all studies (I^2 = 80 % and Cochrane Q at $p< 0.01$).

< Figure 3 about here>

< Figure 4 about here>

< Figure 5 about here>

Comparison of sROC curves in human test samples

Following the bivariate analysis model using the "reitsma" function in mada, we obtained sROC curves comparing the diagnostic performance of the different NAATs in the human test samples (Figure 6). Although the sROC plots overlapped slightly, and the confidence regions significantly so, we conclude from the AUC values that qPCR is the most reliable technique in diagnosing human samples, followed by PCR-ELISA and LAMP, and PCR, in that order.

Through the bivariate meta-regression analysis, we compared the combined effect of sensitivity and false positives in each two techniques 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). This overall outcome of these comparisons was that qPCR performs better than any of the other approaches. These results also imply that the heterogeneity of the results of these primary studies might be explained by the use of different NAATs.

< Figure 6 about here>

Diagnostic accuracy of nucleic acid amplification assays in snails

Our 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.

< Figure 7 about here>

< Figure 8 about here>

< Figure 9 about here>

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 are separated. A significant difference was found when PCR was compared with nPCR through bivariate meta-regression analysis ($p<0.01$). It can, thus, be concluded that PCR is a more reliable test for snail diagnosis than nPCR.

< Figure 10 about here>

Diagnostic accuracy of nucleic acid amplification assays in animals

Although schistosomiasis mansoni is principally a human disease, it is neither 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 material analysed.

< Figure 11 about here>

Discussion

To realize the goal of elimination of schistosomiasis as a public health problem by 2030 as promoted by the World Health Organization (WHO) (WHO, 2020), the use of high-accuracy tests are the highest importance. For this reason, we focused 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 we found minor variations between the techniques investigated, they all performed well for the detection of schistosome DNA in human and snail samples, thus supporting the prerequisite of accurate, sensitive diagnostic tests. Compared with microscopic methods, the sensitivity of the assays examined 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, demonstrated high sensitivity and has 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 true negative cases. Low specificity means presence of a high number of false positives, i.e., more cases that are negative will be diagnosed as positive. The qPCR technique showed the highest specificity with respect to the 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 were somewhat disappointing, in particular nPCR that had the lowest specificity when used to detecting schistosome DNA in snail samples.

The results of the quality assessment using the QUADAS-2 tool showed uncertain risks in most studies, particularly in terms of index test, patient selection as well as 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 random-effects model, heterogeneity was evident for sensitivity, specificity and DOR among the studies investigated. 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 reference index, we noticed that Kato-Katz formalin ether sedimentation (or just ether sedimentation) were the preferential microscopic detection methods used as reference. There were also considerable differences in how the Kato-Katz test was carried out, e.g., one faecal sample using one slide; one faecal sample using 2 slides; three faecal samples using 3 slides; two slides prepared from each of 3 faecal samples; or one faecal sample using 12 slides. We also noticed that different samples (stool, urine, serum, 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 heterogeneity of the diagnosis value.

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

Conclusions

In conclusion, the NAATs have 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, its process complexity and time consumption could limit its usage. LAMP and qPCR are particularly suitable for follow-up research and should have the best potential in large-scale diagnostic surveys. However, the specificity of LAMP needs to be improved.

Data sharing:

Please contact author for data requests.

Author contributions:

HML, ZQQ, MBQ, SX, SL, NX, XNZ, JU, and RB initiated and conceptualized the project. HML, ZQQ, and MBQ investigated the review and collected the data. HML, ZQQ, MBQ, SX, SL and XNZ did the methodology. HML, ZQQ, MBQ, SX, SL and XNZ wrote the original draft, and JU and RB edited and revised the manuscript critically. All authors approved the final version.

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Conflict of interest statement:

The authors declare that they have no competing interests.

Ethical Approval statement:

Not applicable.

References:

Allam AF, Kader O, Zaki A, Shehab AY, Farag HF. Assessing the marginal error in diagnosis and cure of Schistosoma mansoni in areas of low endemicity using Percoll and PCR techniques. Trop Med Int Health 2009;14(3):316-21.
Amaral KB, Silva TP, Malta KK, Carmo LA, Dias FF, Almeida MR, et al. Natural Schistosoma mansoni Infection in the Wild Reservoir Nectomys squamipes Leads to Excessive Lipid Droplet Accumulation in Hepatocytes in the Absence of Liver Functional Impairment. PLoS One 2016;11(11):e0166979.
Archer J, Barksby R, Pennance T, Rostron P, Bakar F, Knopp S, et al. Analytical and Clinical Assessment of a Portable, Isothermal Recombinase Polymerase Amplification (RPA) Assay for the Molecular Diagnosis of Urogenital Schistosomiasis. Molecules 2020;25(18).
Bruscky IS, de Melo FL, de Medeiros ZM, Albuquerque FF, Wanderley LB, da Cunha-Correia C. Nested polymerase chain reaction in cerebrospinal fluid for diagnosing spinal cord schistosomiasis: A promising method. Journal of the neurological sciences 2016;366:87-90.
Carneiro TR, Peralta RH, Pinheiro MC, Oliveira SM, Peralta JM, Bezerra FS. A conventional polymerase chain reaction-based method for the diagnosis of human schistosomiasis in stool samples from individuals in a low-endemicity area. Mem Inst Oswaldo Cruz 2013;108(8):1037-44.
Carvalho GC, Marques LH, Gomes LI, Rabello A, Ribeiro LC, Scopel KK, et al. Polymerase chain reaction for the evaluation of Schistosoma mansoni infection in two low endemicity areas of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 2012;107(7):899-902.
Cochrane. Review Manager. 2021.
https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman. (accessed 20 January 2021).
Colley DG, King CH, Kittur N, Ramzy RMR, Secor WE, Fredericks-James M, et al. Evaluation, Validation, and Recognition of the Point-of-Care Circulating Cathodic Antigen, Urine-Based Assay for Mapping Schistosoma mansoni Infections. Am J Trop Med Hyg 2020;103(1_Suppl):42-9.
Doebler P, Holling H. Meta-Analysis of Diagnostic Accuracy with mada. 2020.
https://cran.r-project.org/web/packages/mada/index.html. (accessed 26 March, 2021)
Enk MJ, Oliveira e Silva G, Rodrigues NB. Diagnostic accuracy and applicability of a PCR system for the detection of Schistosoma mansoni DNA in human urine samples from an endemic area. PLoS One 2012;7(6):e38947.
Espírito-Santo MC, Alvarado-Mora MV, Dias-Neto E, Botelho-Lima LS, Moreira JP, Amorim M, et al. Evaluation of real-time PCR assay to detect Schistosoma mansoni infections in a low endemic setting. BMC Infect Dis 2014;14:558.
Farghaly A, Saleh AA, Mahdy S, El-Khalik DA, El-Aal NFA, Abdel-Rahman SA, et al. Molecular approach for detecting early prepatent Schistosoma mansoni infection in Biomphalaria alexandrina snail host. Journal of Parasitic Diseases 2016;40(3).
Fuss A, Mazigo HD, Tappe D, Kasang C, Mueller A. Comparison of sensitivity and specificity of three diagnostic tests to detect Schistosoma mansoni infections in school children in Mwanza region, Tanzania. PLoS One 2018;13(8):e0202499.
Gandasegui J, Fernández-Soto P, Muro A, Simões Barbosa C, Lopes de Melo F, Loyo R, et al. A field survey using LAMP assay for detection of Schistosoma mansoni in a low-transmission area of schistosomiasis in Umbuzeiro, Brazil: Assessment in human and snail samples. PLoS Negl Trop Dis 2018;12(3):e0006314.
Gentile R, Gonçalves MM, da Costa Neto SF, da Costa MM, Peralta RH, Peralta JM. Evaluation of immunological, parasitological and molecular methods for the diagnosis of Schistosoma mansoni infection before and after chemotherapy treatment with praziquantel in experimentally infected

Nectomys squamipes. Veterinary parasitology 2011;180(3-4):243-9.
Gomes LI, Marques LH, Enk MJ, Coelho PM, Rabello A. Further evaluation of an updated PCR assay for the detection of Schistosoma mansoni DNA in human stool samples. Mem Inst Oswaldo Cruz 2009;104(8):1194-6.
Guegan H, Fillaux J, Charpentier E, Robert-Gangneux F, Chauvin P, Guemas E, et al. Real-time PCR for diagnosis of imported schistosomiasis. PLoS Negl Trop Dis 2019;13(9):e0007711.
Hailegebriel T, Nibret E, Munshea A, Chieffi PP. Prevalence of Schistosoma mansoni and S. haematobium in Snail Intermediate Hosts in Africa: A Systematic Review and Meta-analysis. Journal of Tropical Medicine 2020;2020.
Hamburger J, Abbasi I, Kariuki C, Wanjala A, Mzungu E, Mungai P, et al. Evaluation of loop-mediated isothermal amplification suitable for molecular monitoring of schistosome-infected snails in field laboratories. Am J Trop Med Hyg 2013;88(2):344-51.
Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.2. Cochrane2021. https://www.training.cochrane.org/handbook. (accessed 2 March 2021)
Hotez PJ, Harrison W, Fenwick A, Bustinduy AL, Ducker C, Mbabazi PS, et al. Female genital schistosomiasis and HIV/AIDS: Reversing the neglect of girls and women. Plos Neglected Tropical Diseases 2019;13(4).
Joof E, Andrus PS, Sowunmi K, Onyango VM, Wade CM. Comparing PCR techniques against conventional cercarial shedding methods for detecting Schistosoma mansoni infection in Biomphalaria snails. Acta Tropica 2020;212.
Kebede T, Bech N, Allienne JF, Olivier R, Erko B, Boissier J. Genetic evidence for the role of non-human primates as reservoir hosts for human schistosomiasis. PLoS Negl Trop Dis 2020;14(9):e0008538.
LoVerde PT. Schistosomiasis. Adv Exp Med Biol 2019;1154:45-70.
Magalhães FdC, Resende SD, Senra C, Graeff-Teixeira C, Enk MJ, Coelho PMZ, et al. Accuracy of real-time polymerase chain reaction to detect Schistosoma mansoni – infected individuals from an endemic area with low parasite loads. Parasitology 2020;147(10).
McManus DP, Bergquist R, Cai P, Ranasinghe S, Tebeje BM, You H. Schistosomiasis-from immunopathology to vaccines. Semin Immunopathol 2020;42(3):355-71.
McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN. Schistosomiasis. Nat Rev Dis Primers 2018;4(1):13.
Melo FL, Gomes AL, Barbosa CS, Werkhauser RP, Abath FG. Development of molecular approaches for the identification of transmission sites of schistosomiasis. Trans R Soc Trop Med Hyg 2006;100(11):1049-55.
Meurs L, Brienen E, Mbow M, Ochola EA, Mboup S, Karanja DM, et al. Is PCR the Next Reference Standard for the Diagnosis of Schistosoma in Stool? A Comparison with Microscopy in Senegal and Kenya. PLoS Negl Trop Dis 2015;9(7):e0003959.
Moher D LA, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. Int J Surg 2010.
Mwangi IN, Agola EL, Mugambi RM, Shiraho EA, Mkoji GM, Marchand B. Development and Evaluation of a Loop-Mediated Isothermal Amplification Assay for Diagnosis of Schistosoma mansoni Infection in Faecal Samples. Journal of Parasitology Research 2018;2018.
Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic acids research 2000;28(12):E63.
Nzelu CO, Kato H, Peters NC. Loop-mediated isothermal amplification (LAMP): An advanced molecular point-of-care technique for the detection of Leishmania infection. PLoS Negl Trop Dis 2019;13(11):e0007698.
Ogongo P, Kariuki TM, Wilson RA. Diagnosis of schistosomiasis mansoni: an evaluation of existing methods and research towards single worm pair detection. Parasitology 2018;145(11):1355-66.
Oliveira LM, Santos HL, Gonçalves MM, Barreto MG, Peralta JM. Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low-intensity Schistosoma mansoni infection. Diagnostic microbiology and infectious disease 2010;68(4):416-21.
Pontes LA, Oliveira MC, Katz N, Dias-Neto E, Rabello A. Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with Schistosoma mansoni. Am J Trop Med Hyg 2003;68(6):652-6.

Price M, Cyrs A, Sikasunge CS, Mwansa J, Lodh N. Testing the Infection Prevalence of Schistosoma mansoni after Mass Drug Administration by Comparing Sensitivity and Specificity of Species-Specific Repeat Fragment Amplification by PCR and Loop-Mediated Isothermal Amplification. Am J Trop Med Hyg 2019;101(1):78-83.
PROSPERO. International Prospective Register of Systematic Reviews. 2021. https://www.crd.york.ac.uk/PROSPERO/. (accessed 20 December 2021).
Qin ZQ, Xu J, Feng T, Lv S, Qian YJ, Zhang LJ, et al. Field Evaluation of a Loop-Mediated Isothermal Amplification (LAMP) Platform for the Detection of Schistosoma japonicum Infection in Oncomelania hupensis Snails. Trop Med Infect Dis 2018;3(4).
Richards L, Erko B, Ponpetch K, Ryan SJ, Liang S. Assessing the nonhuman primate reservoir of Schistosoma mansoni in Africa: a systematic review. Infect Dis Poverty 2019;8(1):32.
Sady H, Al-Mekhlafi HM, Ngui R, Atroosh WM, Al-Delaimy AK, Nasr NA, et al. Detection of Schistosoma mansoni and Schistosoma haematobium by Real-Time PCR with High Resolution Melting Analysis. International journal of molecular sciences 2015;16(7):16085-103.
Schunk M, Kebede Mekonnen S, Wondafrash B, Mengele C, Fleischmann E, Herbinger KH, et al. Use of Occult Blood Detection Cards for Real-Time PCR-Based Diagnosis of Schistosoma Mansoni Infection. PLoS One 2015;10(9):e0137730.
Schwarzer G. General Package for Meta-Analysis. 2021. https://CRAN.R-project.org/package=meta. (accessed 20 February 2021)
Schwarzer G, R.Carpenter J, Rucker G. Meta-Analysis with R. In: Gentleman R, Hornik K, Parmigiani G, editors. Meta-Analysis of Diagnostic Test Accuracy Studies. Switzerland: Springer International Publishing; 2015. p. 217-36.
Senra C, Gomes LI, Siqueira LMV, Coelho PMZ, Rabello A, Oliveira E. Development of a laboratorial platform for diagnosis of schistosomiasis mansoni by PCR-ELISA. BMC research notes 2018;11(1):455.
Shim SR, Kim SJ, Lee J. Diagnostic test accuracy: application and practice using R software. Epidemiol Health 2019;41:e2019007.
Siqueira LM, Gomes LI, Oliveira E, Oliveira ER, Oliveira Á A, Enk MJ, et al. Evaluation of parasitological and molecular techniques for the diagnosis and assessment of cure of schistosomiasis mansoni in a low transmission area. Mem Inst Oswaldo Cruz 2015;110(2):209-14.
Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med 2002;21(9):1237-56.
Weerakoon KG, Gordon CA, McManus DP. DNA Diagnostics for Schistosomiasis Control. Trop Med Infect Dis 2018;3(3).
Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155(8):529-36.
WHO. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee. World Health Organization 2002a.
WHO. Prevention and control of schistosomiasis and soiltransmitted helminthiasis. WHO Technical Report Series No 912 Geneva, Switzerland: World Health Organization 2002b.
WHO. Ending the neglect to attain the Sustainable Development Goals--A road map for neglected tropical diseases 2021–2030. 2020.
WHO NTDs. Schistosomiasis: progress report 2001–2011, strategic plan 2012–2020. 2013.
Zhao S, Liu YH, Ye YY, Li W, Zhang JF, Guo LC, et al. Establishment of the gene detection method of Schistosoma mansoni based on the recombinase-aided isothermal amplification assay. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 2020;32(4):335-9.

Figures Legends

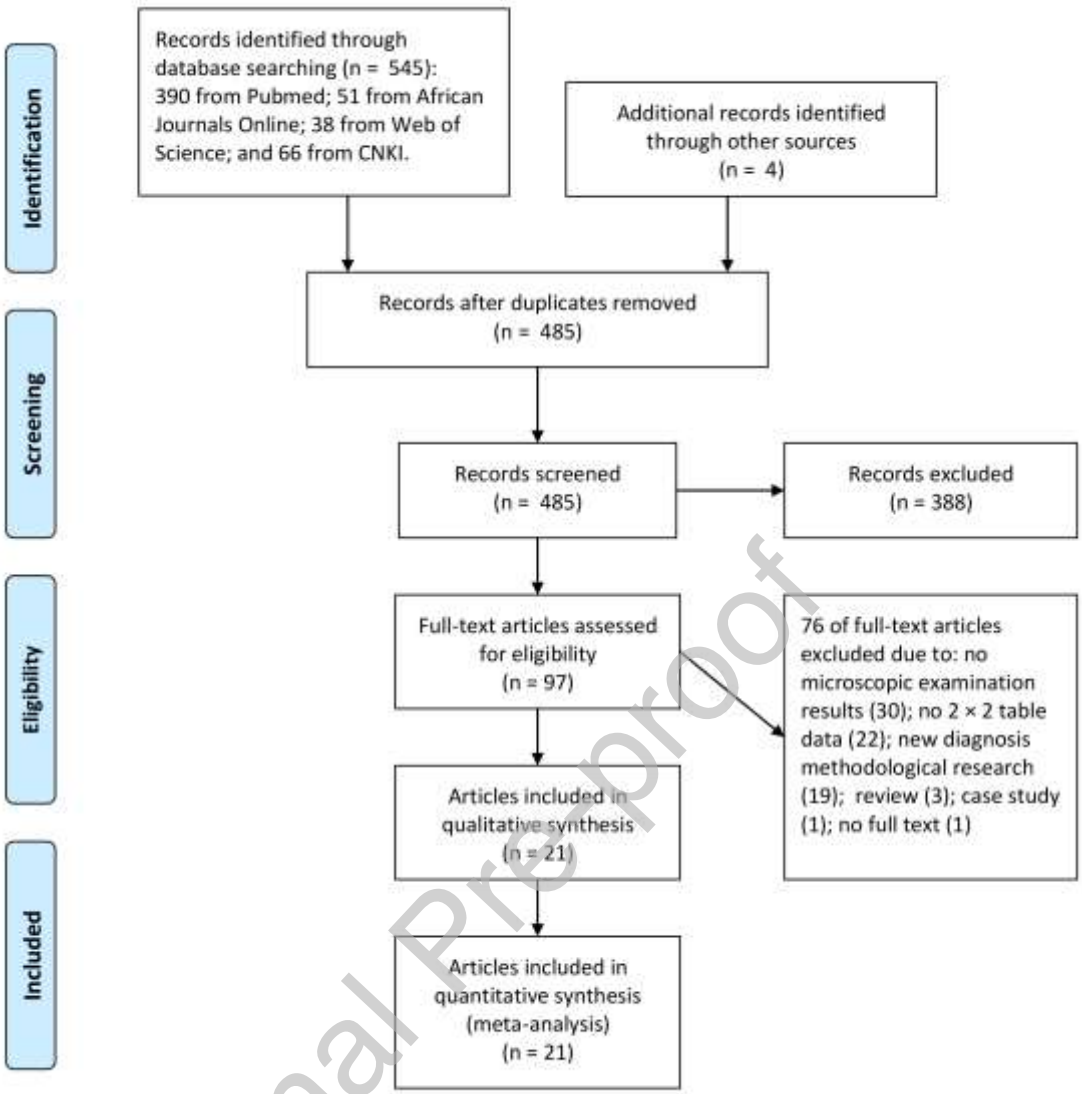

Figure 1. PRISMA flow chart of the article selection (PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement)

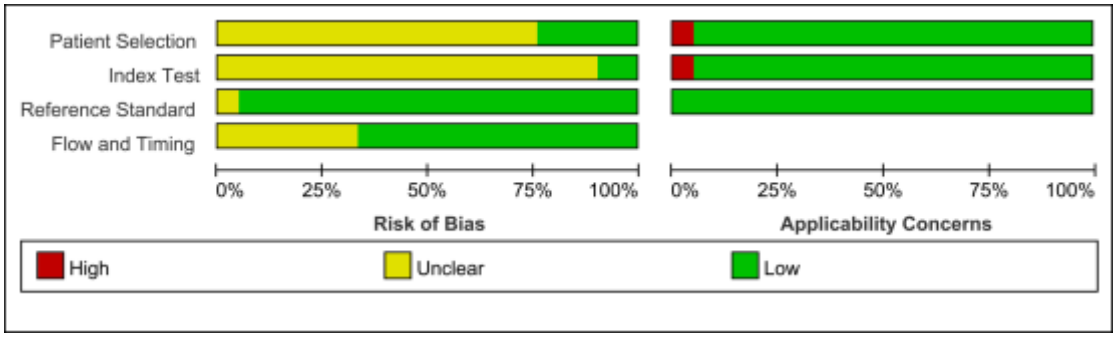

Figure 2. Quality assessment of 21 articles using QUADAS-2 (Proportion represents the summary for risk of bias and applicability. QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies)

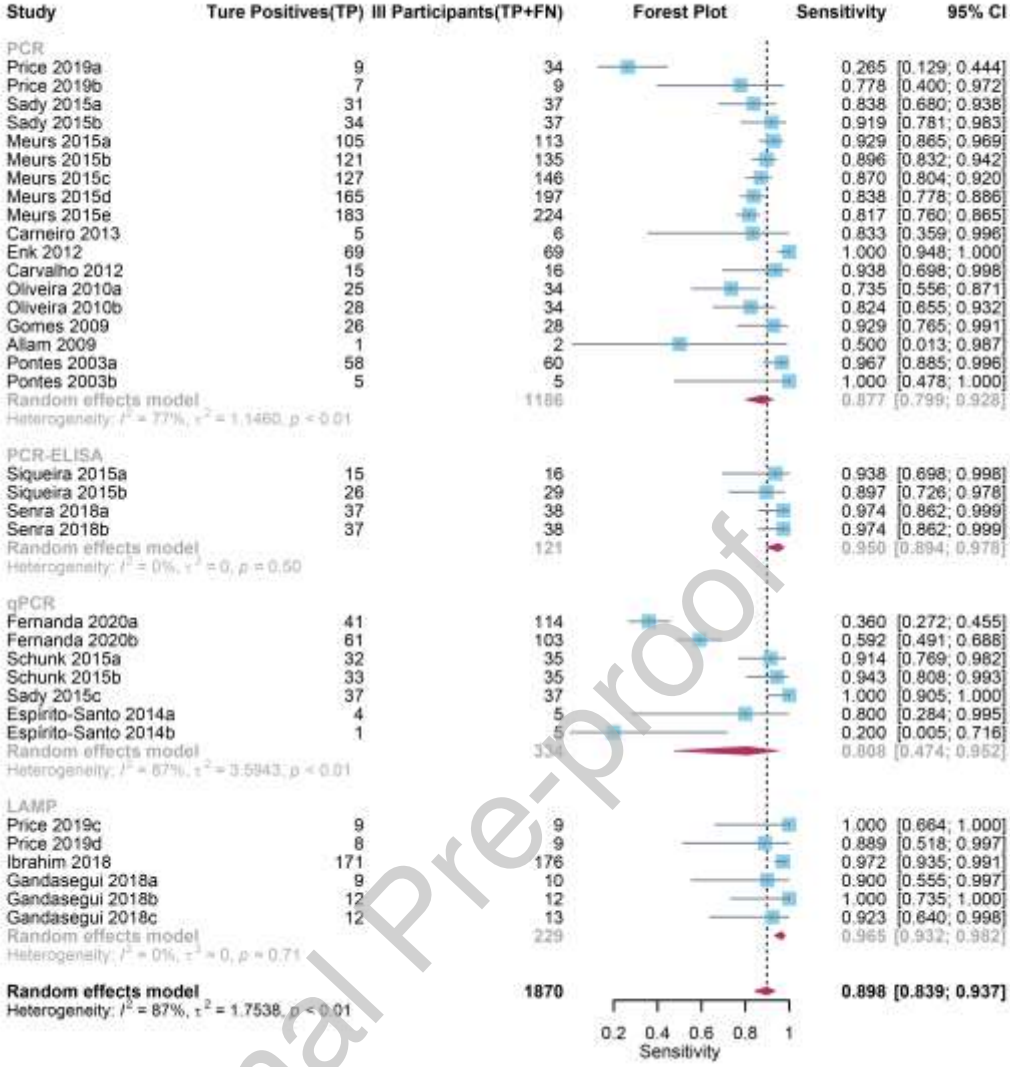

Figure 3. Human studies: forest plots for sensitivity determination (The studies are shown by different NAATs. The sensitivity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.)

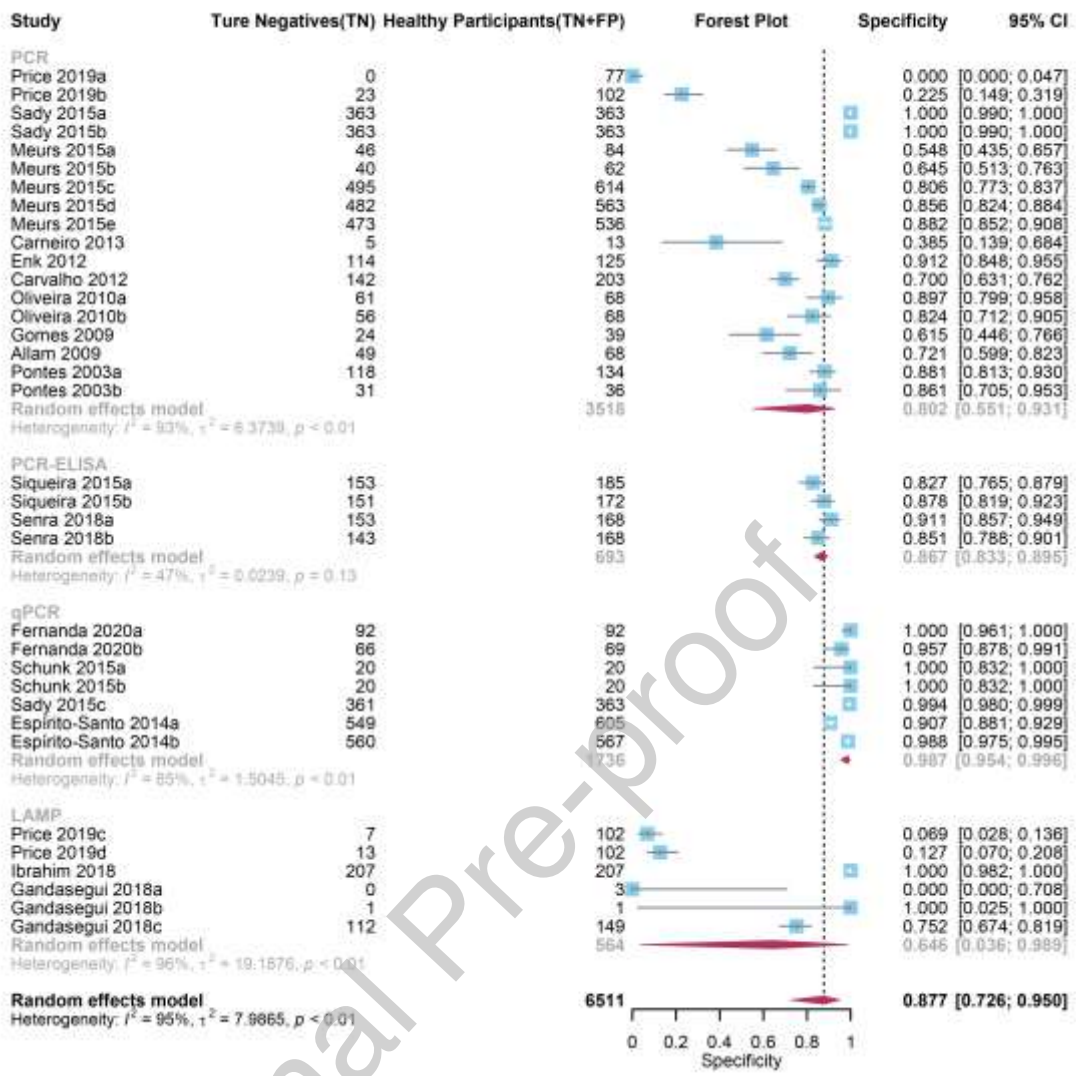

Figure 4. Human studies: forest plots for specificity determination (The studies are shown by different NAATs. The specificity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.)

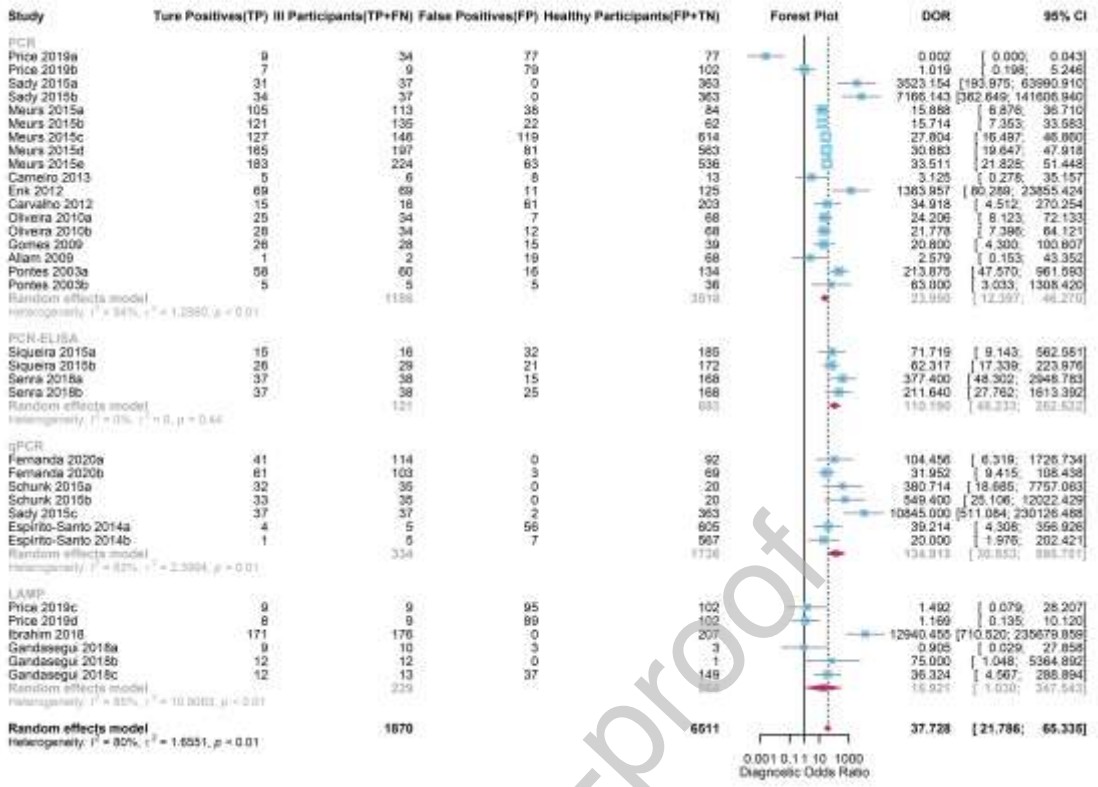

Figure 5. Human studies: forest plots for diagnostic odds ratio determination (The studies are shown by different NAATs. The diagnostic odds ratio and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.)

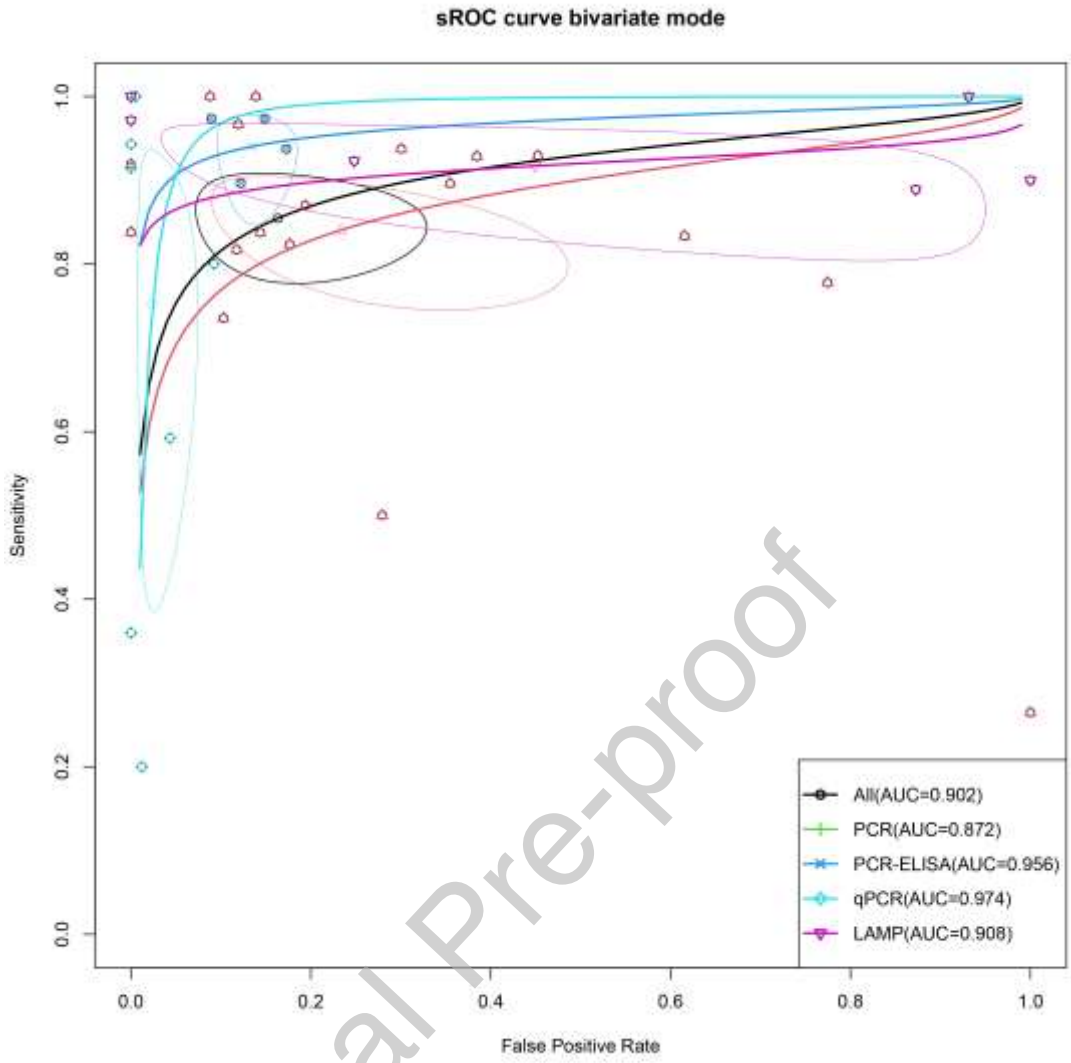

Figure 6. sROC curves and AUC for different NAATs applied for human test samples (Coloured curves are the sROC curves of different NAATs. Coloured dots indicate individual studies. The areas marked as continuous coloured lines represent the confidence region for the point estimate of the pair of sensitivity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

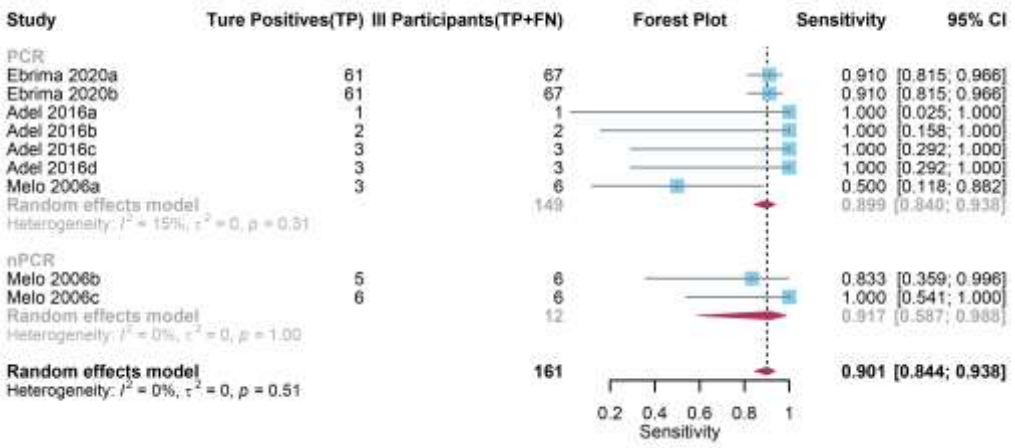

Figure 7. Snail studies: forest plots for sensitivity determination (The studies are shown by different NAATs. The sensitivity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.)

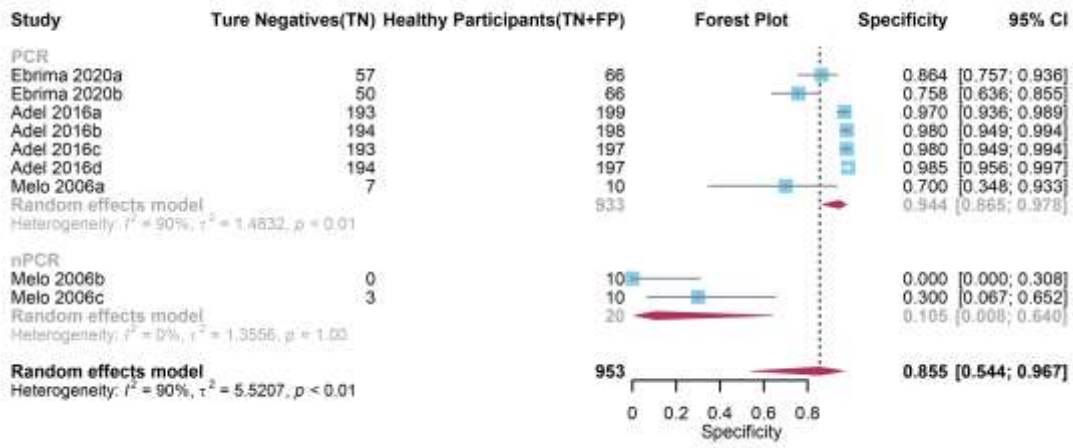

Figure 8. Snail studies: forest plots for specificity determination (The studies are shown by different NAATs. The specificity and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.)

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.)

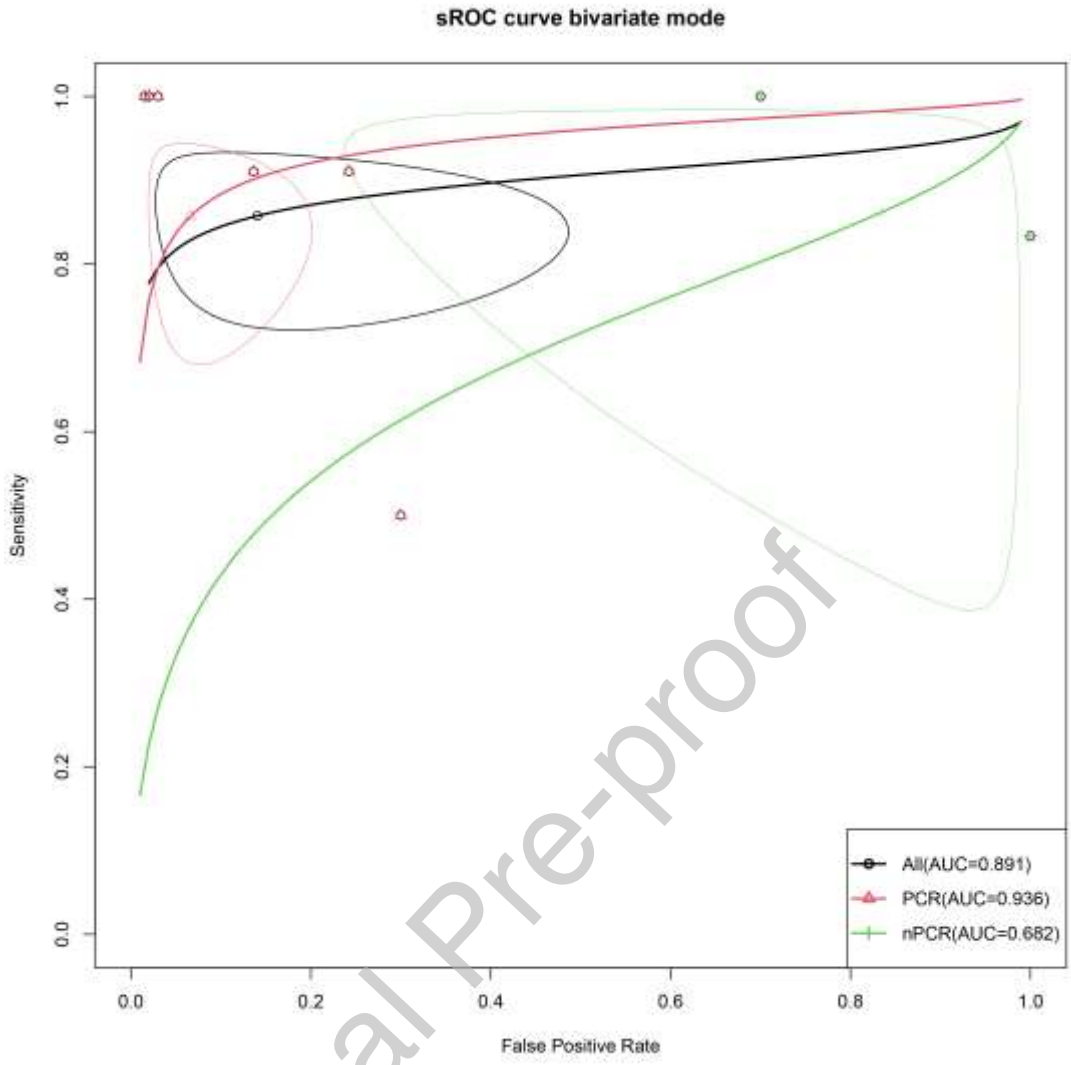

Figure 10. sROC curves of different NAATs in detecting snail samples (Coloured curves are the sROC curves of different NAATs. Coloured dots indicate individual studies. The areas marked as continuous coloured lines represent the confidence region for the point estimate of the pair of sensitivity and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

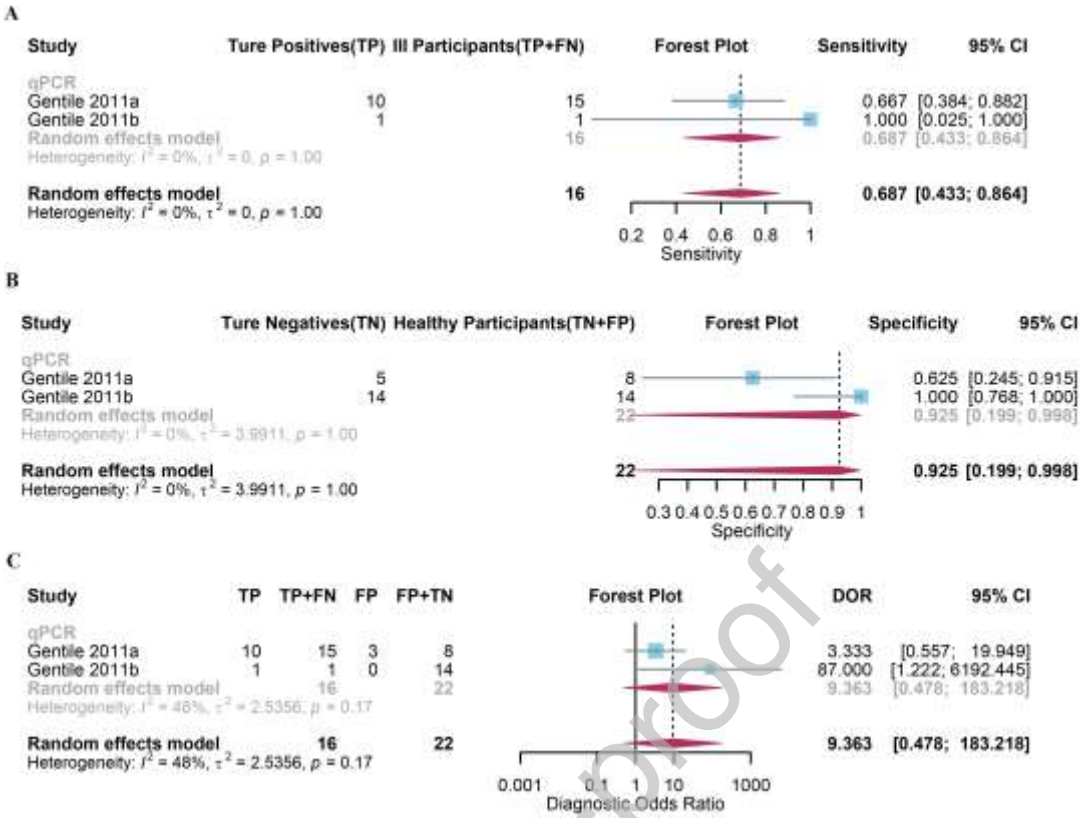

Figure 11 Animal studies: forest plots for sensitivity (A), specificity (B), and diagnostic odds ratio (C) determination (The sensitivity, specificity, diagnostic odds ratio, and 95% confidence interval (95% CI) are displayed for each individual study and pooled estimates.)

Table 1 Characteristics of studies extracted from articles included in 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 inverstigation	Stool (human)	Kato-Katz	PCR
Enk, 2012(Enk et al., 2012)	Brazil	Field inverstigation	Stool (human)[c] Urine (human)[c]	Kato Katz PCR	PCR
Carvalho, 2012(Carvalho et al., 2012)	Brazil	Field inverstigation	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]*N. squamipes*

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		
Anmial	qPCR	1 / 2 / 38	1 / 2 / 38	

* NAATs: Nucleic acid amplifying technique.