THE CURRENT EVIDENCE ON DIAGNOSTIC ACCURACY OF COMMERCIAL BASED NUCLEIC ACID AMPLIFICATION TESTS FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS. A META-REGRESSION ANALYSIS

APPENDIX – DETAILED STUDY SELECTION METHODOLOGY

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LITERATURE SEARCH

We used systematic methods to identify studies analysing diagnostic accuracy of commercial nucleic acid amplification tests (NAATs) for the diagnosis of pulmonary tuberculosis (TB).

An investigator (S.G.) developed a computerized search strategy to identify relevant studies published until 1 March 2005 in the Medline and Embase electronic databases.

This strategy employed key words (both controlled vocabulary and free text terms) and was divided into three parts,. each connected by the [AND] bullion. The first part mapped the search for tuberculosis, the second, more complexpart, mapped the search for nucleic acid amplification tests, while the final part limited the search to English-language studies. A detailed description of our search strategy is shown in fig S1. We first searched for articles in the Medline database. All duplicate articles found in the Embase database were excluded.

We updated the literature search in Medline through 1 July 2005 by employing the same search strategy. Subsequently, the references listed in articles previously retrieved were scrutinized.

FIGURE S1
MEDLINE search strategy for studies on diagnostic capability of commercial NAAT for pulmonary tuberculosis

mycobacterium tuberculosis[MeSH Terms] OR (mycobacterium[Text Word] AND tuberculosis[Text Word]) OR tuberculosis[MeSH Terms] OR tuberculosis[Text Word]

AND

(((((molecular[Text Word] AND ((“diagnostic techniques and procedures”[MeSH Terms]) OR (diagnostic[Text Word] AND (techniques[Text Word] OR tests[Text Word] OR methods[Text Word]))) OR direct[Text Word] AND (detection[Text Word] OR amplification[Text Word] OR identification[Text Word]) AND (test[Text Word] OR assay[Text Word] OR system[Text Word] OR method[Text Word] OR technique[Text Word]) OR nucleic acid amplification techniques[MeSH Terms] OR ((nucleic[Text Word] AND acid[Text Word] AND amplification[Text Word]) OR (molecular[Text Word]) AND (test[Text Word] OR assay[Text Word] OR system[Text Word] OR method[Text Word] OR technique[Text Word]) OR amplification[Text Word] AND (gene[Text Word] OR genes[Text Word] OR genetic[Text Word] OR DNA[Text Word] OR deoxyribonucleic[Text Word] OR RNA[Text Word] OR ribonucleic[Text Word]) OR PCR[Text Word] OR polymerase chain reaction[MeSH Terms] OR (polymerase[Text Word] AND chain[Text Word] AND reaction[Text Word]) OR LCX[Text Word] OR LCR[Text Word] OR (ligase[Text Word] AND chain[Text Word] AND reaction[Text Word]) OR SDA[Text Word] OR (strand[Text Word] AND displacement[Text Word] AND amplification[Text Word] OR BDProbeTec[Text Word] OR MTD[Text Word] OR (mycobacterium [Text Word] AND direct[Text Word] AND test[Text Word]) OR (Amplicor[Text Word]) OR (Cobas[Text Word] AND Amplicor[Text Word]))))))))

AND English[Lang]

STUDY SELECTION PROCESS

To be included, a study had to 1) examine commercial NAAT diagnostic performance on respiratory samples (a percentage of <5% of non respiratory samples was tolerated); 2) apply Mycobacterium tuberculosis (MTB) culture performed on the same sample as reference standard for pulmonary TB diagnosis; 3) provide sufficient original data to permit calculation of sensitivity and/or specificity; and 4) be written in English. We excluded studies 1) reporting sensitivity and specificity "revised" by means of discrepant analysis; 2) analysing sample population used in different studies published by the same research group (only the article reporting on the largest number of samples was considered) and 3) including a percentage of gastric aspirates of >5%.

The selection of the studies to be included in the meta-analysis was based upon the above mentioned criteria. As assessing study relevance and extracting data require the use of judgement, two independent investigators reviewed the articles and disagreements were settled by consensus. The initial search strategy (fig S1) yielded a total of 3,302 citations and a further 41 were identified by checking the references of retrieved articles. A careful review of titles and abstracts eliminated 2,864 citations, that were clearly outside the scope of the meta-analysis. The relevance of the remaining 479 was judged by applying inclusion and exclusion criteria. After eliminating 73 reviews (or comments)[1-73] and 66 articles exploring technical issues related to the use of NAAT both for direct application on clinical specimens and for identification of Mycobacterium tuberculosis in culture[74-139], we evaluated 340 studies reporting primary data on clinical utility of NAAT for TB.

Of the articles which did not analyse commercial NAAT, 171 used different types of home-grown PCR[140-310] and 25 evaluated two commercial NAAT which were withdrawn from the market more than seven years ago, i.e. the old version of Gen Probe MTD and the Q-beta replicase[311-335]. Although the Abbott Ligase Chain Reaction (LCx) test has been withdrawn from the market in 2002, we elected to include articles on LCx performance as this test is still used in many hospital laboratories.

The inclusion of a percentage above 5% of non respiratory samples lead to the exclusion of further 15 articles[336-350]. Although gastric aspirate examination is used for pulmonary TB diagnosis, the lower yield of both culture and NAAT on this type of sample could markedly and variably alter accuracy estimates[343]. Thus, we excluded 7 articles that evaluated a percentage of gastric aspirates higher than 5%[351-357].

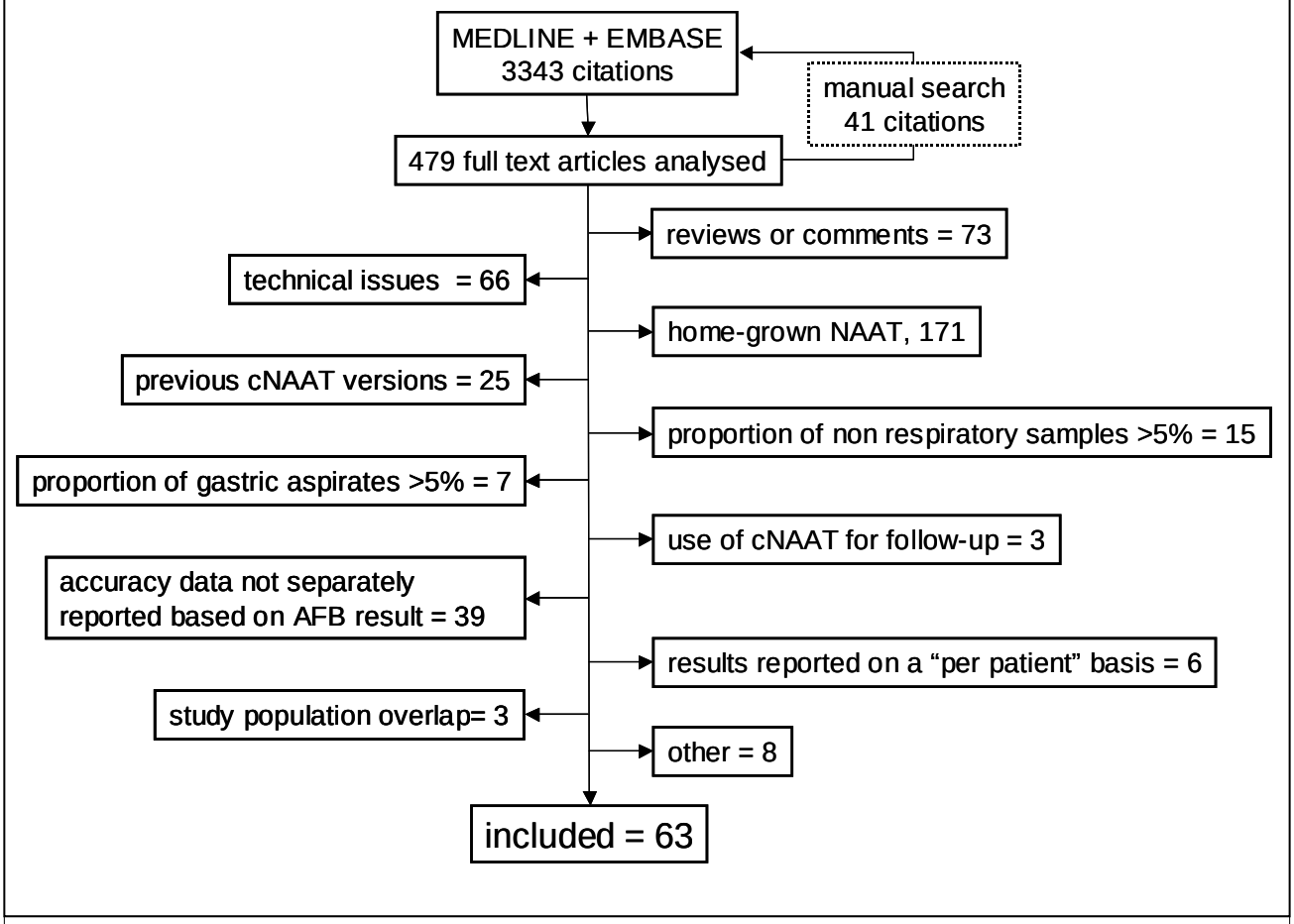
With the exception of 3 articles on commercial NAAT utility for pulmonary TB follow-up[358-360] and 8 articles not fulfilling inclusion criteria for different reasons[361-368], the remaining 114 articles estimated sensitivity and specificity of commercial NAAT for pulmonary TB diagnosis. Among them, 39 did not provide data sufficient for computation of accuracy for AFB+ and/or

AFB- samples[369-407], 3 were from authors publishing several reports on the same samples [408-410] and in 6 articles accuracy was analysed on a “per patient” basis, without taking into consideration the different number of specimens collected for each patient (from 2 to 6)[411-416]. The remaining 63 articles were finally included in the meta-analysis[417-479]. The reasons for exclusion are also outlined in Fig. S2.

Since 8 out of the 63 included articles analyzed 2 different commercial NAATs, 71 studies on the whole were available (56 on AFB+ and 60 on AFB- samples). The commercial NAATs evaluated were: Roche Amplicor MTB (25 studies), its entirely automated version, Cobas Amplicor MTB (10), E-MTD (14), BDProbeTecET (12), LCx (10). Overall, the 63 articles examined 51,160 samples: 5,729 MTB culture positive and 45,431 MTB culture negative. The median number of samples per study was 410 (IQR 247 to 662), with a median pulmonary TB prevalence of 0,14 (IQR 0,07-0,3).

A summary of the 63 included articles reporting the 2x2 table of true positives and negatives and false positives and negatives and the related diagnostic odds ratios is shown in Table S1.

FIGURE S2
Study selection process



Legend.
cNAAT, commercial nucleic acid amplification tests; AFB, acid fast bacilli

Table S1. Results of the 63 included studies

study	NAAT	AFB+ samples						AFB- samples					
		a/c	b/d	TPR	TNR	DOR	prevalence	a/c	b/d	TPR	TNR	DOR	prevalence
Beavis 1995 [420]	Amplicor	80/1	10/8	0,99	0,44	64	0,82	3/3	7/420	0,50	0,98	60	0,01
D'Amato 1995 [432]	Amplicor	13/1	5/17	0,93	0,74	44	0,39	21/20	9/899	0,51	0,99	105	0,04
Moore 1995 [457]	Amplicor	82/1	6/2	0,99	0,25	27	0,91	52/27	8/798	0,66	0,97	75	0,09
Vuorinen 1995 [473]	Amplicor	20/0	0/3	1	1	240	0,87	2/4	2/218	0,33	0,99	55	0,03
Zolnir-Dove 1995 [479]	Amplicor	40/0	3/0	1	0,14	13	0,93	8/2	9/219	0,80	0,99	97	0,04
Bennedsen 1996 [421]	Amplicor	413/39	0/36	0,91	1	762	0,93	123/79	254/6250	0,61	0,96	38	0,03
Bergmann 1996 [423]	Amplicor	40/1	1/10	0,98	0,91	400	0,79	8/12	5/879	0,40	0,99	117	0,02
Cartuyvels 1996 [427]	Amplicor	11/1	1/0	0,92	0,50	6	0,92	6/7	16/614	0,46	0,98	33	0,02
Dalovisio 1996 [431]	Amplicor	25/3	3/34	0,89	0,92	94	0,43	16/12	12/283	0,57	0,96	31	0,09
Dilworth 1996 [435]	Amplicor	11/1	0/1	0,92	1	22	0,92	-	-	-	-	-	-
Ichyiama 1996 [440]	Amplicor	74/0	5/10	1	0,67	296	0,83	45/2	26/257	0,94	0,87	222	0,14
Soini 1996 [469]	Amplicor	12/1	8/4	0,92	0,33	6	0,52	1/1	4/45	0,50	0,92	11	0,04
Tevere 1996 [470]	Amplicor	59/0	1/17	1	0,94	2006	0,77	16/7	4/558	0,70	0,99	319	0,04
Lebrun 1997 [448]	Amplicor	42/1	0/12	0,98	1	1008	0,78	9/17	0/14	0,34	1	14,82	0,64
Liu 1997 [451]	Amplicor	41/0	0/42	1	1	6888	0,49	2/0	0/1	1	1	8	0,57
Piersimoni 1997 [460]	Amplicor	-	-	-	-	-	-	6/1	2/272	0,86	0,99	816	0,02
Smith MB 1997 [467]	Amplicor	23/1	8/14	0,96	0,64	40	0,52	8/4	3/498	0,67	0,97	810	0,02
Yuen 1997 [478]*	Amplicor	48/3	2/3	0,94	0,60	24	0,91	11/8	4/130	0,58	0,97	45	0,12
Kearns 1998 [446]	Amplicor	37/1	2/7	0,97	0,78	130	0,81	8/13	0/24	0,44	1	30	0,46
Gamboa 1998 [438]*	Amplicor	176/0	28/27	1	0,49	339	0,76	24/23	0/477	0,51	1	995	0,09
Miller 2002 [456]	Amplicor	103/2	0/30	0,98	1	3090	0,78	-	-	-	-	-	-
Yee 2002 [477]	Amplicor	-	-	-	-	-	-	3/2	1/66	0,60	0,99	99	0,07
Cleary 2003 [429]	Amplicor	190/8	0/168	0,96	1	7980	0,54	-	-	-	-	-	-
Iinuma 2003 [441]*	Amplicor	-	-	-	-	-	-	6/4	0/304	0,60	1	912	0,03
Iwamoto 2003 [442]	Amplicor	12/0	2/13	1	0,87	156	0,44	8/0	1/30	1	0,97	480	0,21
Gamboa 1998 [438]*	Cobas Amplicor	176/0	28/27	1	0,49	339	0,76	28/19	0/477	0,60	1	1406	0,09
Jan 1998 [443]	Cobas Amplicor	19/2	0/7	0,91	1	133	0,75	15/4	4/537	0,79	0,99	503	0,03
Reischl 1998 [463]	Cobas Amplicor	42/2	1/10	0,95	0,91	210	0,80	6/7	4/571	0,46	0,99	122	0,02
Yam 1998 [476]	Cobas Amplicor	18/1	0/7	0,95	1	252	0,73	20/5	0/334	0,80	1	2672	0,07
Wang 1999 [475]*	Cobas Amplicor	-	-	-	-	-	-	5/1	6/152	0,83	0,96	127	0,04
Scarparo 2000 [466]*	Cobas Amplicor	95/2	0/11	0,98	1	1045	0,90	13/4	5/166	0,76	0,96	108	0,09
Bogard 2001 [426]	Cobas Amplicor	183/16	8/42	0,92	0,84	60	0,80	95/36	47/4650	0,73	0,99	261	0,03
Jonsson 2003 [445]	Cobas Amplicor	46/1	0/17	0,98	1	1564	0,73	42/19	6/746	0,69	0,99	275	0,08
Levidiotou 2003 [450]	Cobas Amplicor	189/6	15/40	0,97	0,73	84	0,78	21/38	30/7215	0,36	1	133	0,01
Kim 2004 [447]*	Cobas Amplicor	-	-	-	-	-	-	6/7	0/126	0,46	1	216	0,09
Bergmann 2000 [422]	BDProbeTecET	12/0	0/11	1	1	91	0,52	2/2	6/567	0,50	0,99	95	0,01
Barrett 2002 [419]	BDProbeTecET	99/2	2/64	0,98	0,97	1584	0,60	1/7	1/29	0,13	0,97	4	0,21
Johansen 2002 [444]	BDProbeTecET	85/0	0/11	1	1	3740	0,89	39/26	3/186	0,60	0,98	93	0,26
Maugein 2002 [453]	BDProbeTecET	43/0	0/6	1	1	1032	0,88	18/8	8/464	0,69	0,98	131	0,05
Piersimoni 2002 [462]*	BDProbeTecET	75/1	0/31	0,99	1	4650	0,71	11/4	2/207	0,73	1	285	0,07
De la Calle 2003 [433]	BDProbeTecET	21/0	0/1	1	1	84	0,95	9/2	2/442	0,82	0,99	995	0,02
Iinuma 2003 [441]*	BDProbeTecET	-	-	-	-	-	-	5/5	1/303	0,50	1	303	0,03
Mazzarelli 2003 [454]	BDProbeTecET	133/2	1/27	0,99	0,96	1796	0,83	35/14	6/343	0,71	0,98	143	0,12
McHugh 2004 [455]	BDProbeTecET	48/0	2/0	1	0,20	24	0,96	33/2	28/245	0,94	0,90	149	0,11
Kim 2004 [447]*	BDProbeTecET	-	-	-	-	-	-	7/6	7/119	0,54	0,94	18	0,10
Rusch-Gerd. 2004 [465]	BDProbeTecET	36/0	0/2	1	1	288	0,95	54/8	36/612	0,87	0,94	106	0,09
Wang 2004 [474]	BDProbeTecET	11/1	8/1	0,92	0,11	1,4	0,57	11/7	27/486	0,61	0,95	29	0,04
Bodmer 1996 [425]	E-MTD	14/0	0/19	1	1	1064	0,42	6/4	5/669	0,60	0,99	201	0,01
Gamboa 1998 [437]	E-MTD	48/0	0/19	1	1	3648	0,72	42/5	0/296	0,89	1	4973	0,14
Piersimoni 1998 [461]*	E-MTD	36/0	0/6	1	1	864	0,86	24/1	6/175	0,96	0,97	700	0,12
Bergmann 1999 [424]	E-MTD	13/0	2/7	1	0,78	528	0,59	15/8	10/949	0,65	0,99	178	0,02
Chedore 1999 [428]	E-MTD	189/0	7/286	1	0,98	15444	0,39	5/0	2/127	1	0,98	635	0,04
Smith MB 1999 [468]	E-MTD	14/1	0/7	0,93	1	196	0,68	5/0	13/243	1	0,99	77	0,02
Gallina 2000 [436]	E-MTD	75/6	5/15	0,93	0,75	37	0,80	27/9	68/155	0,75	0,70	7	0,14
Scarparo 2000 [466]*	E-MTD	89/8	0/11	0,92	1	245	0,90	13/4	6/165	0,76	0,96	89	0,09
Alcala 2001 [417]	E-MTD	77/2	1/2	0,98	0,67	77	0,96	28/8	39/506	0,78	0,93	45	0,06
Gurkan 2002 [439]	E-MTD	-	-	-	-	-	-	10/6	72/297	0,63	0,80	7	0,04
O'Sullivan 2002 [459]	E-MTD	30/0	1/61	1	0,98	3660	0,33	3/1	2/238	0,75	0,99	357	0,02
Piersimoni 2002 [462]*	E-MTD	71/5	2/29	0,93	0,94	206	0,71	10/5	0/209	0,67	1	836	0,07
Coll 2003 [430]	E-MTD	184/1	1/47	1	0,98	8648	0,79	52/23	3/2993	0,69	1	2256	0,02
Lemaitre 2004 [449]	E-MTD	-	-	-	-	-	-	3/5	1/20	0,38	0,95	12	0,28
Ausina 1997 [418]	LCx	139/2	20/26	0,99	0,57	90	0,75	15/16	3/299	0,48	0,99	93	0,09
Yuen 1997 [478]*	LCx	47/4	4/1	0,92	0,20	3	0,91	7/12	7/127	0,37	0,95	11	0,12
Denis 1998 [434]	LCx	7/0	0/1	1	1	28	0,88	2/0	4/196	1	0,98	196	0,01
Moore 1998 [458]	LCx	13/0	8/0	1	0,06	2	0,62	12/9	21/451	0,57	0,98	73	0,04
Piersimoni 1998 [461]*	LCx	33/3	0/6	0,92	1	132	0,86	16/9	6/175	0,64	0,97	56	0,12
Lumb 1999 [452]	LCx	65/1	10/63	0,99	0,86	410	0,47	27/26	12/1879	0,51	0,99	163	0,03
Rohner 1999 [464]	LCx	55/3	1/9	0,95	0,90	165	0,85	14/6	44/1869	0,70	0,98	99	0,01
Viveiros 1999 [472]	LCx	20/0	3/5	1	0,63	67	0,71	8/1	3/53	0,89	0,95	141	0,14
Wang 1999 [475]*	LCx	-	-	-	-	-	-	6/0	7/151	1	0,96	258	0,03
Viinanen 2000 [471]	LCx	23/1	0/9	0,96	1	414	0,73	3/4	5/202	0,43	0,98	30	0,03

Legend to Table 1S

*, articles analysing two different commercial NAATs; a, true positives; c, false negatives; b, false positives; d, true negatives; TPR, true positive rate (sensitivity); TNR, true negative rate (specificity); DOR, diagnostic odds ratio. Prevalence was calculated as the proportion of Mycobacterium tuberculosis culture positive samples among AFB+ or AFB- sample population. In parentheses, reference number.

EVALUATION OF THE PUBLICATION BIAS

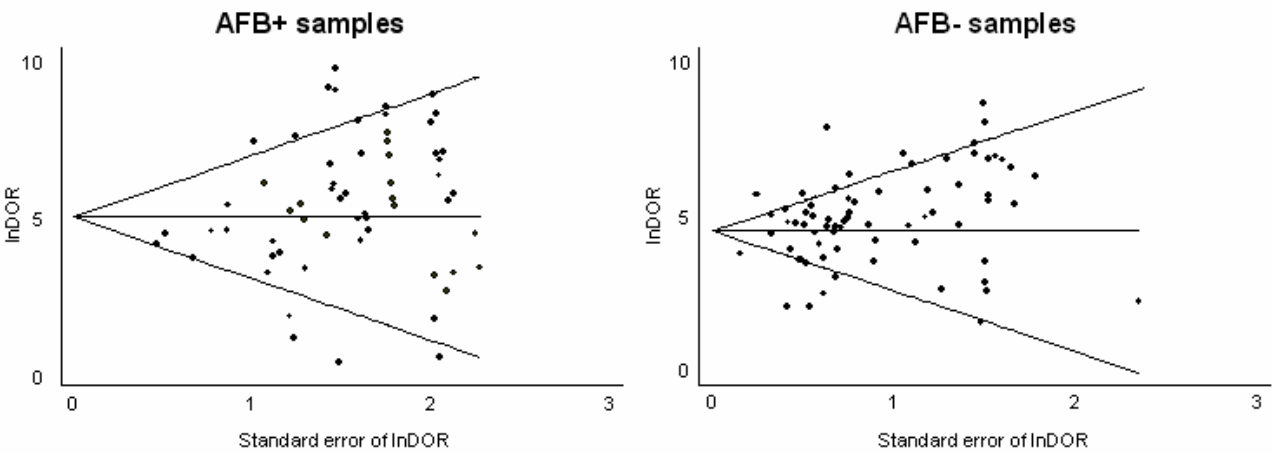
Publication bias is the tendency on the part of investigators to publish (and of reviewers to accept for publication) manuscripts with more optimistic results. As meta-analysis collects the body of published studies on a specified topic, it may reinforce the impact of the selection of positive papers and report inflated pooled estimates of diagnostic accuracy[480].

We used a funnel plot to evaluate primary studies for publication bias. In a funnel plot the diagnostic odds ratio (DOR) is plotted against a measure of its precision, such as standard error (that is inversely proportional to sample size). As random variation of DOR estimates are expected to be smaller among larger studies, while results from small studies be widely scattered, meta-analysis data should be symmetrically distributed in a funnel shaped area. If publication bias is present, i.e., a tendency of small studies to report positive results (or, better, small studies with negative results are lacking) is present an asymmetrical funnel plot will be obtained. The Begg and Mazumdar adjusted rank correlation test (between DOR and its variances)[481] and the Egger regression asymmetry test[482], are used for the statistical analysis of the visual funnel graph.

In our case, although the Begg's test was not significant (AFB+ samples, p=0,202; AFB- samples, p=0,170), the visual inspection of both funnel plots revealed the presence of some asymmetry (figure S3) and the Egger's test indicated a significant correlation between lnDOR and its standard error both for studies on AFB+ samples (regression coefficient 1,14, p=0.011) and for studies on AFB- samples (regression coefficient 0,97 p=0.022).

FIGURE S3

Funnel plot with pseudo 95% confidence limits.



Legend.
LnDOR, logarithm of Diagnostic Odds Ratio. Each closed circle represents each study in the meta-analysis, while the line in the center represents the summary value of lnDOR

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