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Diagnostic Performance of ELISA versus PCR in Detecting Toxoplasma gondii in Cases of Spontaneous Abortion: A Systematic Review and Meta-Analysis

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ABSTRACT:

Background. Toxoplasma gondii infection in pregnancy is a recognised cause of fetal loss, but laboratories investigating spontaneous abortion use either serology (ELISA for IgM and IgG) or PCR, and the two often disagree. We synthesised published studies that applied both tests to the same women with spontaneous abortion to compare their positivity and agreement.

Methods. We searched PubMed, Europe PMC, Google Scholar, OpenAlex, Crossref and publisher sites from inception to 3 October 2026. Eligible studies tested women with spontaneous abortion by ELISA and by PCR on blood, placenta or abortion tissue. Positivity was pooled with binomial–normal random-effects models. Where studies cross-tabulated the two tests, we estimated sensitivity, specificity, diagnostic odds ratio (DOR) and Cohen's κ of IgM- and IgG-ELISA against PCR as the reference.

Results. Eighteen studies (2,224 women; Iran 11, Iraq 3, Egypt 2, Libya 1, Mexico 1) were included. Pooled positivity was 6.5% (95% CI 4.2–9.9%) for IgM-ELISA, 29.8% (26.1–33.7%) for IgG-ELISA and 7.7% (5.1–11.6%) for PCR, with high heterogeneity ($I^2 \approx 91\%$). Within studies, PCR positivity matched IgM positivity (ratio 1.07, 0.83–1.38) but was about one-quarter of IgG positivity (0.28, 0.15–0.56). Against PCR, IgM-ELISA had a sensitivity of 64.8% (32.5–87.6%), specificity of 99.1% (94.5–99.8%), DOR of 92.8 and κ of 0.69 (substantial agreement); 31% of PCR-positive women were IgM-negative. IgG-ELISA had a sensitivity of 53.9%, specificity of 65.4%, DOR of 2.2 (0.6–8.3) and κ of 0.09. PCR positivity was higher in the four Arab-country studies than in Iranian studies (29.1% vs 3.8%; $P < 0.001$), although most of the Arab-country studies had reporting concerns.

Conclusions. IgM-ELISA and PCR classify a similar proportion of women as positive and agree substantially, but neither captures all cases; IgG-ELISA reflects past exposure and should not be used to attribute an abortion to T. gondii. A combined approach — maternal IgM (with avidity) plus PCR — is suggested, although it has not been evaluated directly. The evidence base is small, regionally concentrated and of variable quality, and a standardised multicentre diagnostic study is needed.

1. INTRODUCTION

Toxoplasma gondii is a ubiquitous protozoan that infects an estimated one-third of the world's population [1, 2]. Seroprevalence among women of childbearing age varies several-fold between regions [3], and the pooled global seroprevalence of latent infection in pregnant women is about one-third [4]. Primary infection during pregnancy can be transmitted to the fetus, with a risk that rises with gestational age while the severity of fetal damage falls [5]. Congenital toxoplasmosis is estimated to cause about 190,000 cases worldwide each year [6].

Early-pregnancy infection can end in miscarriage. Systematic reviews have linked T. gondii infection to spontaneous abortion and other adverse pregnancy outcomes [7–9]. In a 2021 meta-analysis, T. gondii DNA was found in about 10% of women with spontaneous abortion, and recent infection was associated with higher odds of miscarriage [8]. Determining whether a particular abortion was associated with toxoplasmosis matters for counselling, for the management of subsequent pregnancies and for public-health surveillance.

The laboratory diagnosis is not straightforward [10–12]. ELISA detection of IgG shows that a woman has been infected at some time, which in endemic regions is common and usually remote. IgM suggests recent infection, but it can persist for months or years and false-positive results occur, so IgM alone overestimates acute infection [13, 14]. IgG avidity testing helps to date infection [15, 16]. PCR detects parasite DNA directly, using multicopy targets such as the B1 gene or the 529-bp repetitive element (RE) [17–19]. Its yield depends on the specimen (maternal blood, placenta or fetal tissue), the parasite load, the timing of sampling and the assay format.

Many laboratories, particularly in the Middle East and North Africa, have applied both ELISA and PCR to women with spontaneous abortion. Individual studies have reached conflicting conclusions about which test performs better, and they are small. Earlier meta-analyses pooled seroprevalence or PCR positivity separately [7, 8]; none, to our knowledge, has quantified how the two methods agree when applied to the same women.

This systematic review and meta-analysis therefore asked three questions: (i) what proportion of women with spontaneous abortion are positive by IgM-ELISA, IgG-ELISA and PCR; (ii) how closely do ELISA and PCR agree within the same cohorts; and (iii) how accurate are IgM- and IgG-ELISA when PCR is taken as the reference for active infection?

2. METHODS

2.1 Protocol and reporting

The review followed the PRISMA 2020 statement and its extension for diagnostic test accuracy reviews (PRISMA-DTA) [20, 21]. The protocol was not prospectively registered.

2.2 Eligibility criteria

We included primary studies, of any design, that (i) enrolled women with spontaneous abortion (miscarriage, early pregnancy loss or abortion products); (ii) tested those women for T. gondii by ELISA on maternal serum (IgM and/or IgG, or class not specified), including enzyme-linked fluorescent assays; (iii) tested the same women by PCR of any format (conventional, nested, semi-nested or real-time) on maternal blood, placenta, products of conception or fetal tissue; and (iv) reported, or allowed exact derivation of, the number of positive results for each test. Studies were excluded if they used only rapid, latex or immunofluorescence serology; only non-PCR amplification such as LAMP; enrolled ongoing pregnancies or mixed populations from which the abortion subgroup could not be separated; or were reviews, animal studies or duplicate reports of the same cohort.

2.3 Search strategy

We searched PubMed, Europe PMC, Google Scholar, OpenAlex and Crossref, and publisher and journal websites, from inception to 3 October 2026, without language restrictions. The core query was: (Toxoplasma OR toxoplasmosis) AND (abortion OR miscarriage OR "pregnancy loss" OR placenta OR "products of conception") AND (PCR OR "polymerase chain reaction" OR molecular) AND (ELISA OR "enzyme-linked" OR IgM OR IgG OR serolog*). Searches were repeated with country names for regions with many publications (Iran, Iraq, Egypt, Turkey, the Arab states, South Asia, Africa, Latin America). Reference lists of previous meta-analyses were checked [7, 8].

2.4 Study selection and data extraction

Titles and abstracts were screened, and potentially eligible reports were retrieved and assessed in full where accessible. For each study we extracted bibliographic data, country and city, design, number of women with spontaneous abortion tested, ELISA kit, PCR specimen, format and target gene, and the counts of IgM-, IgG- and PCR-positive women. Where a study reported serology by mutually exclusive groups (IgG only, IgM only, both), totals were summed. Counts reported only as percentages were back-calculated, and the back-calculation was checked against every other figure in the paper. Where serostatus was cross-tabulated against PCR, we built 2 × 2 tables with PCR as the reference standard. Extracted counts were checked against the source documents in a second verification pass, and discrepancies were resolved by re-reading the source; for three studies some items could be checked only at abstract level.

2.5 Quality assessment

Risk of bias was assessed with the QUADAS-2 domains [22], using pre-specified rules. Patient selection was rated unclear unless consecutive or complete enrolment was stated. Index test (ELISA) was rated low risk when the kit was named, and unclear otherwise. Reference standard was rated high risk for all studies, because PCR is an imperfect reference for active infection [12]. Flow and timing was rated high risk when PCR was not applied to the whole cohort. We also recorded reporting concerns: values reconstructed from percentages, internal inconsistencies, and publication in journals outside the biomedical field or with documented quality concerns.

2.6 Statistical analysis

Positivity for IgM, IgG and PCR was pooled using a binomial–normal (logit) random-effects model fitted by maximum likelihood with Gauss–Hermite quadrature. This model uses the exact binomial likelihood and accommodates studies with zero events without continuity corrections. Study-level 95% CIs used the Wilson method [23]. Heterogeneity was quantified with Cochran's Q and I^2 from the DerSimonian–Laird model on the logit scale [24–26], and we report 95% prediction intervals based on a t-distribution with $k - 2$ degrees of freedom.

The within-study comparison of PCR with IgM and with IgG positivity used log ratios of proportions pooled by DerSimonian–Laird with Hartung–Knapp confidence intervals, which perform better with few, heterogeneous studies [27, 28]. Because each comparison used the same women, ignoring the pairing makes these intervals conservative.

For studies with 2 × 2 tables, sensitivity (ELISA-positive among PCR-positive), specificity (ELISA-negative among PCR-negative), and positive and negative predictive values were each pooled with univariate binomial–normal models. We also fitted the bivariate model of Reitsma et al. [29], but with six or seven studies the between-study correlation went to its boundary (±1), so univariate estimates are presented as primary. Log diagnostic odds ratios (0.5 added to all cells of tables with a zero) and Cohen's κ [30] were pooled by DerSimonian–Laird with Hartung–Knapp intervals; κ was interpreted using the Landis–Koch categories [31].

Subgroup analyses of PCR positivity examined specimen (placenta or fetal tissue vs maternal blood), PCR format (nested or real-time vs conventional or not reported), PCR target (RE-529 vs B1 vs other) and region (Iran vs Arab countries; the single Mexican study was excluded from this comparison). Between-subgroup differences were tested with Q-tests on DerSimonian–Laird logit estimates; subgroup estimates shown are from the binomial–normal model. Small-study effects were examined with a funnel plot and Egger's regression test [32], which is unreliable for proportions [33]; Deeks' test [34] was not performed because fewer than ten studies contributed 2 × 2 tables. Sensitivity analyses repeated the pooling (i) excluding studies with venue concerns or internal inconsistencies, (ii) excluding values reconstructed from percentages, (iii) restricted to full-text-verified studies with directly reported counts and no venue concerns, and (iv) leaving out one study at a time (PCR positivity). Analyses were performed in Python 3.11 (NumPy, SciPy).

3. RESULTS

3.1 Study selection and characteristics

Fifty-two reports were sought for retrieval. Nine could not be retrieved or verified, and 25 of the 43 assessed were excluded (Figure 1): 11 used ELISA without PCR, four used PCR without ELISA, two used only rapid or latex serology, one used

LAMP, two did not study a spontaneous-abortion cohort, two reported no extractable counts, and three were duplicate or companion reports of included cohorts.

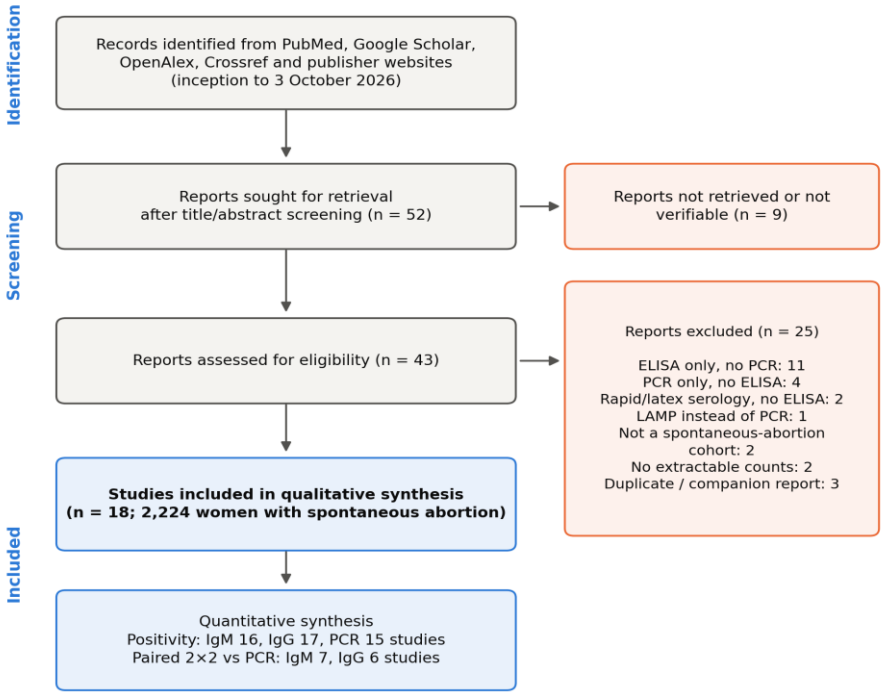


Figure 1. PRISMA 2020 flow diagram of study selection.

The 18 included studies were published between 2013 and 2025 and together tested 2,224 women with spontaneous abortion (Table 1) [35–52]. Eleven were from Iran, three from Iraq, two from Egypt, one from Libya and one from Mexico. Sample sizes ranged from 10 to 480. PCR was performed on placenta or fetal/abortion tissue in nine studies and on maternal blood (with or without placenta) in nine. Nested, semi-nested or real-time PCR was used in 12 studies, and the RE-529 and B1 targets in seven and five studies, respectively. In three studies, PCR was performed only on seropositive women [39, 49, 51]; these contributed to the serology estimates but not to PCR positivity or accuracy. Seven studies cross-tabulated IgM against PCR, and six cross-tabulated IgG against PCR.

Table 1. Characteristics of the 18 included studies (women with spontaneous abortion tested by both ELISA and PCR). Values are n (%).

Study	Country (city)	n	PCR specimen	PCR format / target	ELISA kit	IgM+	IgG+	PCR+
Eslamirad 2014 [35]	Iran (Arak)	87	Fetal/abortion tissue	Conventional / NR	NR	—	34 (39.1)	0 (0.0)
Ghasemi 2016 [36]	Iran (Tehran)	82	Placenta	Conventional / RE-529	NR	3 (3.7)	22 (26.8)	6 (7.3)
Matin 2017 [37]	Iran (Ardabil)	200	Placenta	Nested / RE-529	Biokit	21 (10.5)	99 (49.5)	21 (10.5)
Arefkhah 2019 [38]	Iran (Yasuj)	100	Maternal blood	Real-time / B1	NR	3 (3.0)	7 (7.0)	3 (3.0)
Saki 2021	Iran (Ahvaz)	480	Blood/placenta/cord	Nested / B1	NovaLisa	25	123	Seropositives

Study	Country (city)	n	PCR specimen	PCR format / target	ELISA kit	IgM+	IgG+	PCR+
[39]						(5.2)	(25.6)	only
Khademi 2022 [40]	Iran (southern Iran)	50	Placenta	Conventional / RE-529	NR	0 (0.0)	14 (28.0)	9 (18.0)
Hosseini 2023 [41]	Iran (Mazandaran)	100	Fetal/abortion tissue	Nested / NR	NR	1 (1.0)	68 (68.0)	1 (1.0)
Zeinali 2023 [42]	Iran (Urmia)	215	Fetal/abortion tissue	Nested / RE-529	Pishtaz Teb	3 (1.4)	70 (32.6)	3 (1.4)
Alhubaish 2025 [43]	Iran (Tehran)	120	Maternal blood	Real-time / B1	NovaLisa	2 (1.7)	21 (17.5)	2 (1.7)
Arbabi 2025 [44]	Iran (Zahedan)	163	Placenta	Nested / B1	Pishtaz Teb	12 (7.4)	19 (11.7)	7 (4.3)
Asfaram 2025 [45]	Iran (Meshkin-Shahr)	10	Blood + placenta	Conventional / RE-529	Pishtaz Teb	2 (20.0)	5 (50.0)	3 (30.0)
Vado-Solis 2013 [52]	Mexico (Yucatán)	100	Maternal blood	Conventional / NR	NR	20 (20.0)	55 (55.0)	19 (19.0)
Gashout 2016 [51]	Libya (Tripoli)	140	Blood/serum	Semi-nested / SAG2	Human GmbH	18 (12.9)	48 (34.3)	Seropositives only
Abdel-Aziz 2020 [49]	Egypt (Sohag)	90	Blood + placenta (seropositives only)	Nested / NR	NR	5 (5.6)	29 (32.2)	Seropositives only
El-Sayad 2021 [47]	Egypt (Alexandria)	100	Placenta	NR / NR	NR	46 (46.0)	50 (50.0)	46 (46.0)
Mahmood 2021 [48]	Iraq (Kirkuk)	63	Maternal blood	Real-time / RE-529	NR	12 (19.0)	25 (39.7)	7 (11.1)
Alkardhi 2022 [46]	Iraq (Al-Diwaniyah)	94	Placenta	Nested / RE-529	Sunlong	16 (17.0)	31 (33.0)	30 (31.9)
Madi 2022 [50]	Iraq (Baghdad)	30	Maternal blood	Nested / B1	NR	—	—	10 (33.3)

NR, not reported; RE-529, 529-bp repetitive element. Madi 2022 reported ELISA positivity without antibody class (15/30). Ghasemi 2016: abortion subgroup of a mixed abortion/stillbirth cohort. Mahmood 2021: recurrent abortion.

3.2 Positivity of IgM-ELISA, IgG-ELISA and PCR

Pooled IgM positivity was 6.5% (95% CI 4.2–9.9%; 16 studies, 189/2,107 women), IgG positivity 29.8% (26.1–33.7%; 17 studies, 720/2,194) and PCR positivity 7.7% (5.1–11.6%; 15 studies, 167/1,514) (Figure 2, Table 2). Heterogeneity was high for all three (I^2 91%), and prediction intervals were wide: 0.6–45.5% for IgM, 6.9–70.9% for IgG and 0.2–75.1% for PCR.

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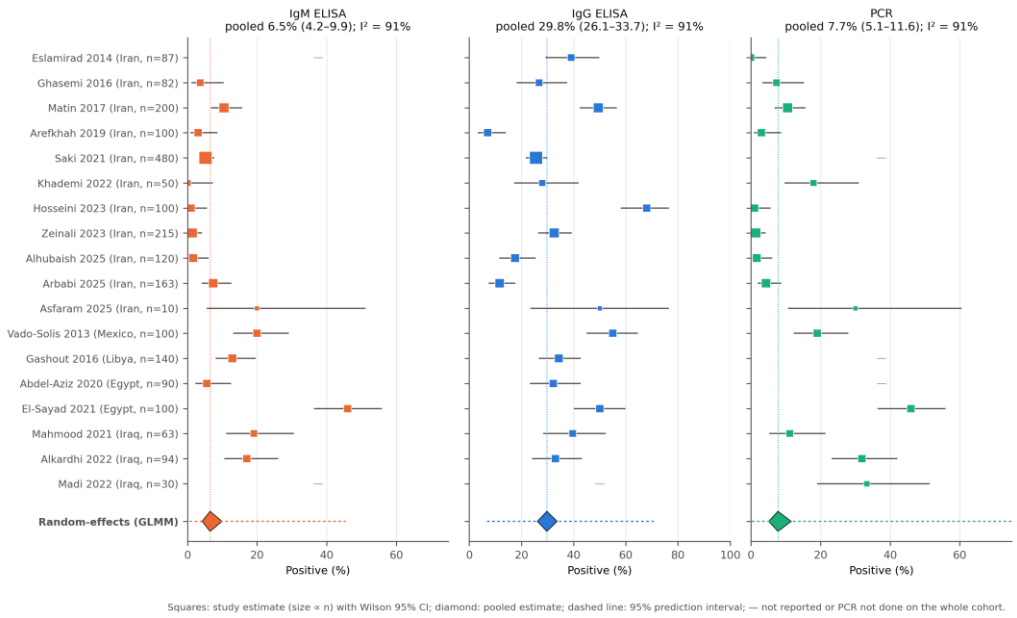


Figure 2. Forest plots of the proportion of women with spontaneous abortion positive by IgM-ELISA, IgG-ELISA and PCR. Squares show study estimates (size proportional to n) with Wilson 95% CIs; diamonds show binomial–normal random-effects pooled estimates; dashed lines show 95% prediction intervals. A dash marks a test not reported, or PCR not performed on the whole cohort.

Within studies, the proportion positive by PCR was close to the proportion positive by IgM (pooled ratio 1.07, 95% CI 0.83–1.38; 13 studies; I^2 10%), but much lower than the proportion positive by IgG (0.28, 0.15–0.56; 14 studies; I^2 86%) (Figure 3). In several Iranian cohorts, IgG positivity exceeded 20% while PCR and IgM positivity stayed below 10%. In the Egyptian and Iraqi cohorts with cross-tabulations, PCR positivity approached IgG positivity (Figure 3) [46, 47].

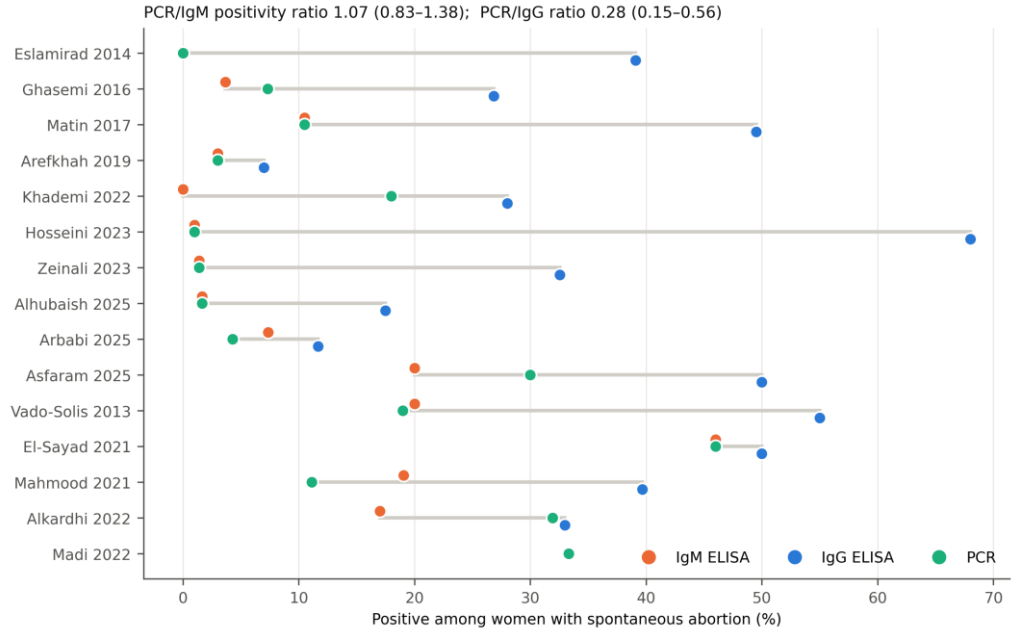


Figure 3. Within-study comparison of IgM-ELISA, IgG-ELISA and PCR positivity. Each row is one cohort; markers are slightly offset vertically so that overlapping values remain visible. Ratios are pooled within-study ratios of PCR to antibody positivity (Hartung–Knapp 95% CI).

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Table 2. Pooled positivity of IgM-ELISA, IgG-ELISA and PCR among women with spontaneous abortion (binomial–normal random-effects model).

Test	Studies	Positive / tested	Pooled % (95% CI)	95% prediction interval	I^2 (%)	τ^2 (logit)
IgM-ELISA	16	189 / 2,107	6.5 (4.2–9.9)	0.6–45.5	91	1.29
IgG-ELISA	17	720 / 2,194	29.8 (26.1–33.7)	6.9–70.9	91	0.67
PCR	15	167 / 1,514	7.7 (5.1–11.6)	0.2–75.1	91	2.70

Within-study ratio of PCR to IgM positivity: 1.07 (0.83–1.38; 13 studies; I^2 10%). PCR to IgG positivity: 0.28 (0.15–0.56; 14 studies; I^2 86%).

3.3 Agreement and accuracy of ELISA against PCR

Seven studies (674 women) provided IgM-versus-PCR 2 × 2 tables, with 114 PCR-positive and 560 PCR-negative women (Table 3, Figure 4). Pooled sensitivity of IgM-ELISA was 64.8% (95% CI 32.5–87.6%; I^2 66%) and specificity 99.1% (94.5–99.8%; I^2 45%). The pooled positive predictive value was 88.1% (76.7–94.4%) and the negative predictive value 95.2% (87.5–98.3%). The DOR was 92.8 (38.4–224.0; I^2 0%), and agreement beyond chance was substantial ($\varkappa$ 0.69, 0.50–0.88; six studies). Across the pooled tables, 35 of 114 PCR-positive women (31%) were IgM-negative, whereas only 11 of 90 IgM-positive women (12%) were PCR-negative. Sensitivity varied from 0% in a cohort with no IgM-positive women and nine PCR-positive placentas [40] to 100% in a cohort with two PCR-positive women [43]. Restricted to the three studies with directly reported cells and no venue concerns [37, 38, 45], sensitivity was 74.1% (54.7–87.1%), specificity 97.9% (95.4–99.0%) and $\varkappa$ 0.73 (0.66–0.80).

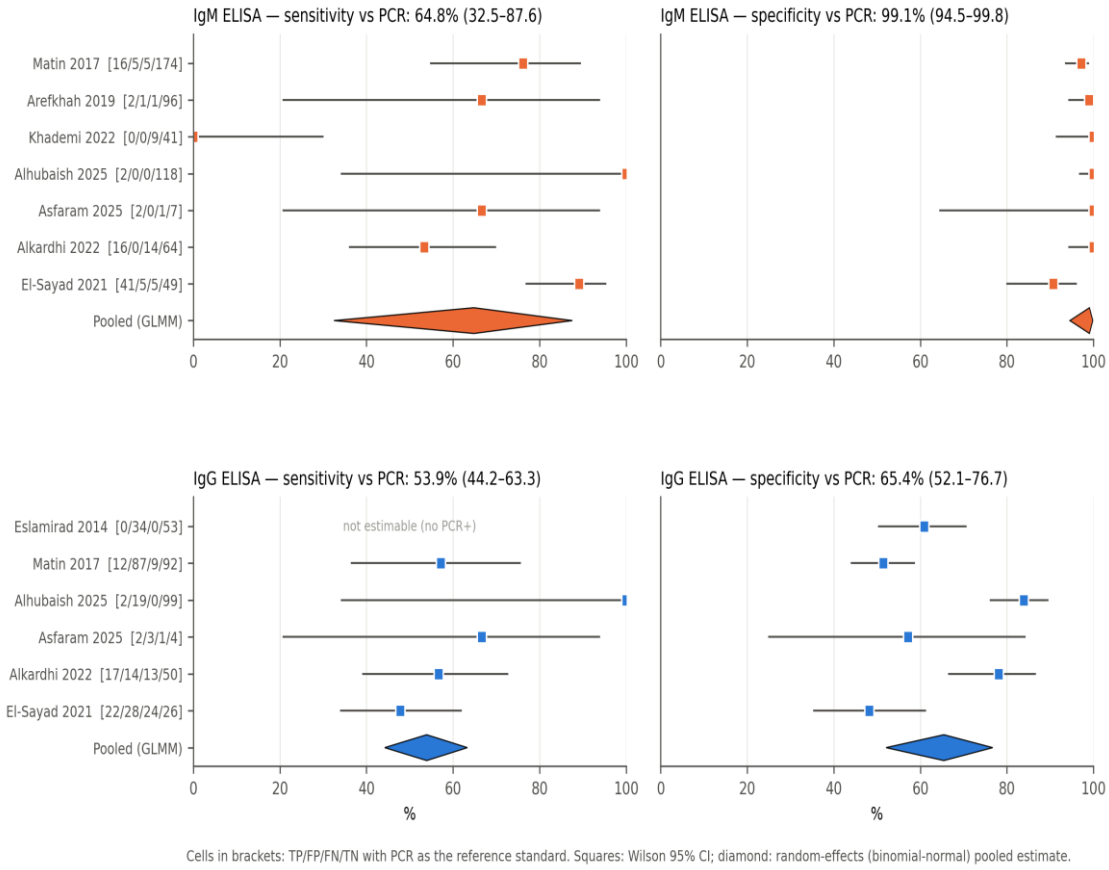


Figure 4. Sensitivity and specificity of IgM-ELISA (top) and IgG-ELISA (bottom) against PCR as the reference standard. Cells are TP/FP/FN/TN; squares are study estimates with Wilson 95% CIs; diamonds are pooled random-effects estimates.

IgG-ELISA performed poorly against PCR. In six studies (611 women), pooled sensitivity was 53.9% (44.2–63.3%; five studies with PCR-positive women), specificity 65.4% (52.1–76.7%; I^2 88%), positive predictive value 17.1% (6.3–38.8%) and DOR 2.2 (0.6–8.3). Agreement did not exceed chance ($\varkappa$ 0.09, −0.07 to 0.26). In the largest cross-tabulated cohort, nine of 21 PCR-positive placentas came from IgG-negative women, and 87 of 99 IgG-positive women had PCR-negative placentas [37]. In ROC space, IgM study estimates clustered in the upper-left corner, while IgG estimates lay close to the chance diagonal (Figure 5).

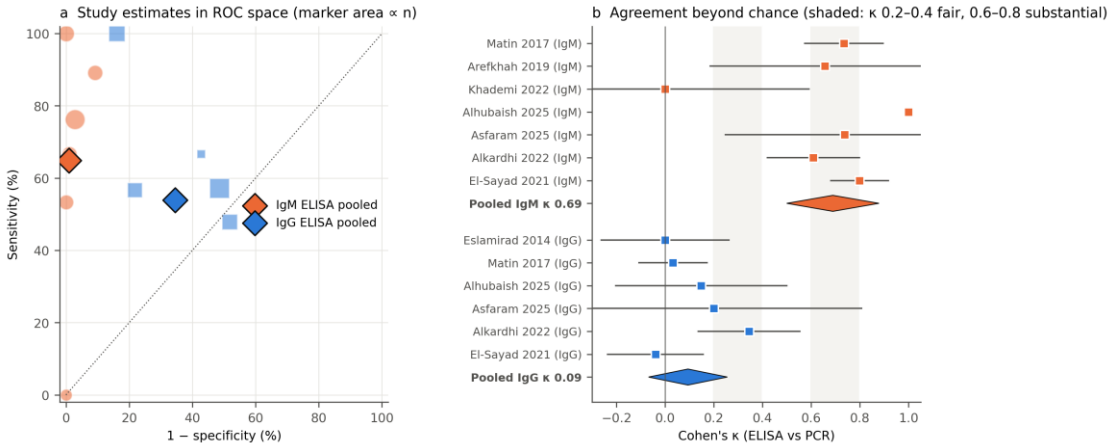


Figure 5. (a) Study-level sensitivity against 1 − specificity for IgM-ELISA (circles) and IgG-ELISA (squares) versus PCR; diamonds mark pooled estimates and the dotted line is chance. (b) Cohen's $\varkappa$ for ELISA–PCR agreement with 95% CIs and pooled estimates (Hartung–Knapp); shaded bands mark the 'fair' (0.2–0.4) and 'substantial' (0.6–0.8) Landis–Koch categories.

The bivariate model gave similar specificity but a lower IgM sensitivity (IgM sensitivity 55.8%, specificity 99.7%; IgG sensitivity 55.7%, specificity 65.5%), but its between-study correlation lay on the boundary of the parameter space, so these are not interpreted further. One Mexican cohort analysed with a Bayesian latent-class model, which does not treat any test as perfect, estimated the sensitivity and specificity of IgM-ELISA at 0.670 and 0.948, of IgG-ELISA at 0.915 and 0.568, and of PCR on maternal blood at 0.747 and 0.981 (uniform priors) [53]. A further Iraqi study reported an ELISA-by-PCR table that was inconsistent with its own serology counts and was therefore not pooled [48].

Table 3. IgM- and IgG-ELISA against PCR as the reference standard: study-level 2 × 2 tables and pooled estimates.

Antibody	Study	TP	FP	FN	TN	Sensitivity % (95% CI)	Specificity % (95% CI)	$\varkappa$
IgM	Matin 2017 [37]	16	5	5	174	76 (55–89)	97 (94–99)	0.73
	Arefkhah 2019 [38]	2	1	1	96	67 (21–94)	99 (94–100)	0.66
	Khademi 2022 [40]	0	0	9	41	0 (0–30)	100 (91–100)	0.00
	Alhubaish 2025 [43]	2	0	0	118	100 (34–100)	100 (97–100)	1.00
	Asfaram 2025 [45]	2	0	1	7	67 (21–94)	100 (65–100)	0.74
	Alkardhi 2022 [46]	16	0	14	64	53 (36–70)	100 (94–100)	0.61
	El-Sayad 2021	41	5	5	49	89 (77–95)	91 (80–96)	0.80

Antibody	Study	TP	FP	FN	TN	Sensitivity % (95% CI)	Specificity % (95% CI)	ϰ
	[47]							
	Pooled	79	11	35	549	**64.8 (32.5–87.6)**	**99.1 (94.5–99.8)**	**0.69 (0.50 to 0.88)**
IgG	Eslamirad 2014 [35]	0	34	0	53	—	61 (50–71)	0.00
	Matin 2017 [37]	12	87	9	92	57 (37–76)	51 (44–59)	0.03
	Alhubaish 2025 [43]	2	19	0	99	100 (34–100)	84 (76–89)	0.15
	Asfaram 2025 [45]	2	3	1	4	67 (21–94)	57 (25–84)	0.20
	Alkardhi 2022 [46]	17	14	13	50	57 (39–73)	78 (67–86)	0.34
	El-Sayad 2021 [47]	22	28	24	26	48 (34–62)	48 (35–61)	-0.04
	Pooled	55	185	47	324	**53.9 (44.2–63.3)**	**65.4 (52.1–76.7)**	**0.09 (-0.07 to 0.26)**

TP, ELISA+/PCR+; FP, ELISA+/PCR−; FN, ELISA−/PCR+; TN, ELISA−/PCR−. Pooled cells are simple sums, for orientation only. IgM: PPV 88.1 (76.7–94.4)%, NPV 95.2 (87.5–98.3)%, DOR 92.8 (38.4–224.0). IgG: PPV 17.1 (6.3–38.8)%, NPV 95.6 (90.7–98.0)%, DOR 2.2 (0.6–8.3). ϰ for Alhubaish 2025 (IgM) is 1.00 with zero standard error and was not pooled. Sensitivity not estimable for Eslamirad 2014 (no PCR-positive women).

3.4 Heterogeneity, subgroups and small-study effects

Region explained the most heterogeneity in PCR positivity: 3.8% (1.7–8.2%) in ten Iranian studies versus 29.1% (17.2–44.8%) in four studies from Arab countries ($P < 0.001$; Figure 6b, Table 4). Three of these four studies had reporting concerns (derived counts or venue concerns). Nested or real-time PCR gave lower pooled positivity than conventional or unspecified formats (5.2% vs 11.5%; $P = 0.093$). Neither specimen type (placenta or fetal tissue 7.1% vs maternal blood 10.5%; $P = 0.555$) nor PCR target (RE-529 11.2%, B1 5.4%; $P = 0.661$) explained heterogeneity.

The funnel plot of PCR positivity was asymmetric (Egger intercept −4.97, $P = 0.005$; Figure 6a). On the logit scale the standard error of a proportion depends on the proportion itself, so less precise studies were mainly those with low positivity; this asymmetry is therefore largely an artefact of the method and cannot be interpreted as small-study or publication bias [33]. By sample size, smaller studies tended towards higher positivity, but not significantly. Leaving out one study at a time moved pooled PCR positivity between 5.2% and 8.6%. Excluding studies with venue concerns or internal inconsistencies lowered PCR positivity to 4.8% (2.1–10.7%; 11 studies), leaving IgM at 5.8% and IgG at 29.4%. Excluding values reconstructed from percentages gave 7.5% for PCR (seven studies), and restricting to full-text-verified studies with directly reported counts and no venue concerns gave 8.0% (three studies) (Table 4).

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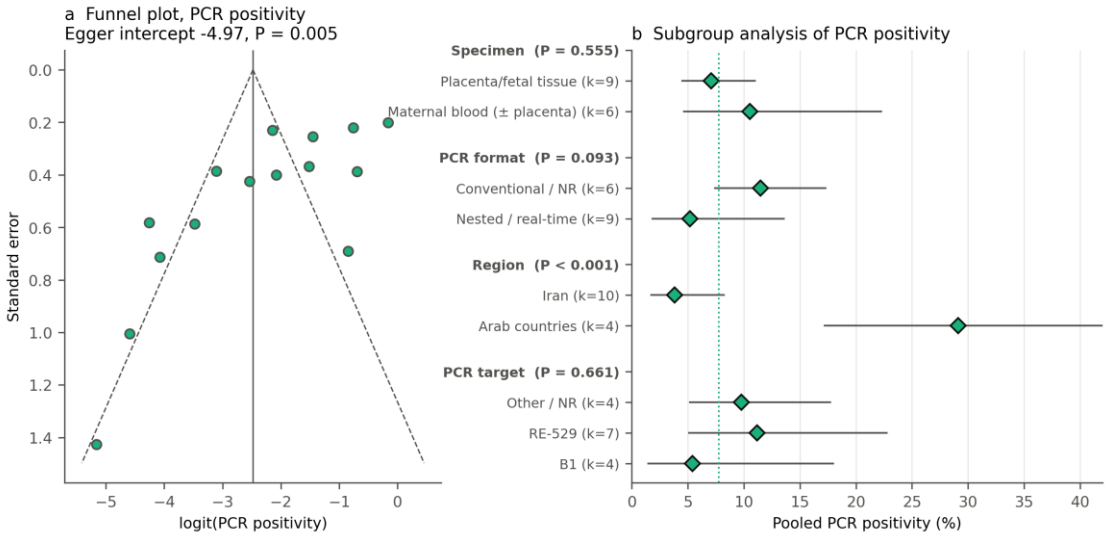


Figure 6. (a) Funnel plot of logit PCR positivity against its standard error, with pseudo-95% limits around the pooled estimate. (b) Subgroup analyses of pooled PCR positivity; the dotted line is the overall pooled estimate.

Table 4. Subgroup and sensitivity analyses of pooled positivity.

Analysis	Subgroup	Studies	Pooled % (95% CI)	P (between groups)
PCR positivity by specimen	Placenta/fetal tissue	9	7.1 (4.5–11.0)	0.555
	Maternal blood (± placenta)	6	10.5 (4.6–22.3)	
PCR positivity by PCR format	Conventional / NR	6	11.5 (7.4–17.3)	0.093
	Nested / real-time	9	5.2 (1.9–13.6)	
PCR positivity by region	Iran	10	3.8 (1.7–8.2)	< 0.001
	Arab countries	4	29.1 (17.2–44.8)	
PCR positivity by PCR target	Other / NR	4	9.8 (5.2–17.7)	0.661
	RE-529	7	11.2 (5.1–22.7)	
	B1	4	5.4 (1.5–18.0)	
Sensitivity: high-confidence studies only	IgM	6	6.6 (3.6–11.7)	
	IgG	6	35.1 (28.3–42.5)	
	PCR	3	8.0 (4.2–14.8)	
Sensitivity: excluding questionable venues/inconsistent data	IgM	12	5.8 (3.0–10.8)	
	IgG	13	29.4 (26.0–33.1)	
	PCR	11	4.8 (2.1–10.7)	
Sensitivity: excluding values reconstructed from percentages	IgM	10	6.7 (3.9–11.4)	

Analysis	Subgroup	Studies	Pooled % (95% CI)	P (between groups)
	IgG	10	36.8 (32.6–41.3)	
	PCR	7	7.5 (4.6–12.2)	
Leave-one-out (PCR)	range of pooled estimates	14 each	5.2–8.6	

Egger's test for PCR positivity: intercept -4.97 (SE 1.46), P = 0.005.

3.5 Risk of bias and reporting quality

All studies carried a high risk of bias in the reference-standard domain, because PCR is an imperfect gold standard. Patient selection was unclear in all studies, and three had high risk in the flow-and-timing domain because PCR was restricted to seropositive women (Table 5). In eight studies at least some counts had to be derived from percentages or reported accuracy statistics, eight were assessed from the abstract alone because the full text was inaccessible, and three were published in journals with documented quality concerns or outside the biomedical field. Ten of 18 studies did not name the ELISA kit, and five did not report the PCR target.

Table 5. Risk of bias (QUADAS-2 domains, applied with pre-specified rules) and reporting concerns.

Study	Patient selection	Index test (ELISA)	Reference standard (PCR)	Flow and timing	Applicability concern	Reporting concerns
Eslamirad 2014	Unclear	Unclear	High	Low	None noted	abstract only, derived counts
Ghasemi 2016	Unclear	Unclear	High	Low	Subgroup of mixed abortion/stillbirth cohort	abstract only, derived counts
Matin 2017	Unclear	Low	High	Low	None noted	None
Arefkhah 2019	Unclear	Unclear	High	Low	None noted	abstract only
Saki 2021	Unclear	Low	High	High (PCR on seropositives only)	None noted	None
Khademi 2022	Unclear	Unclear	High	Low	None noted	abstract only, derived counts
Hosseini 2023	Unclear	Unclear	High	Low	None noted	abstract only
Zeinali 2023	Unclear	Low	High	Low	None noted	None
Alhubaish 2025	Unclear	Low	High	Low	None noted	derived counts, venue concern
Arbabi 2025	Unclear	Low	High	Low	None noted	derived counts
Asfaram 2025	Unclear	Low	High	Low	Very small (n = 10)	None
Vado-Solis 2013	Unclear	Unclear	High	Low	None noted	abstract only, internal

Study	Patient selection	Index test (ELISA)	Reference standard (PCR)	Flow and timing	Applicability concern	Reporting concerns
						inconsistency
Gashout 2016	Unclear	Low	High	High (PCR on seropositives only)	None noted	None
Abdel-Aziz 2020	Unclear	Unclear	High	High (PCR on seropositives only)	None noted	None
El-Sayad 2021	Unclear	Unclear	High	Low	None noted	abstract only, derived counts
Mahmood 2021	Unclear	Unclear	High	Low	Recurrent abortion only	derived counts, venue concern, internal inconsistency
Alkardhi 2022	Unclear	Low	High	Low	None noted	venue concern
Madi 2022	Unclear	Unclear	High	Low	None noted	abstract only, derived counts

Rules: patient selection rated unclear unless consecutive or complete enrolment was stated in an accessible report; index test low risk when the ELISA kit was named; reference standard high risk for all because PCR is an imperfect reference; flow and timing high risk when PCR was not performed on the whole cohort.

4. DISCUSSION

4.1 Principal findings

This meta-analysis brings together 18 studies that applied ELISA and PCR to the same 2,224 women with spontaneous abortion. Three findings stand out. First, IgM-ELISA and PCR were positive in a similar proportion of women (about 6–8%), whereas IgG-ELISA was positive in about 30%. Second, when cross-tabulated, IgM and PCR agreed substantially ($\varkappa$ 0.69), with high specificity and a DOR near 93, but about one-third of PCR-positive women were IgM-negative. Third, IgG positivity carried almost no information about whether parasite DNA was present ($\varkappa$ 0.09, DOR 2.2). These results argue against attributing an abortion to T. gondii on the basis of IgG alone, and suggest that a marker of recent infection should be combined with direct detection of the parasite.

4.2 Interpretation

The close match between IgM and PCR positivity is biologically plausible. Both reflect recent or active infection, whereas IgG persists for life after any past exposure [1, 11]. In regions where 30–50% of women are IgG-positive, IgG cannot separate infection that preceded pregnancy, which carries little risk to the fetus, from infection acquired during pregnancy [3, 4]. The PCR-negative results among IgG-positive women in this review are consistent with chronic, pre-conceptional infection.

The incomplete overlap between IgM and PCR has several explanations. IgM-positive, PCR-negative women may have had residual IgM from an infection months before conception [13, 14], parasitaemia below the PCR detection limit, or parasites in tissue not sampled. IgM-negative, PCR-positive women may have been tested before IgM had appeared, or after it had declined, or may represent reactivation, false-positive PCR from contamination (a particular risk with nested assays), or low-level DNA without clinical significance [12, 19]. Placental or abortion tissue gave lower pooled positivity than maternal blood, although the difference was not significant; parasite distribution in placenta is patchy, and small tissue samples may miss it. The latent-class analysis from Mexico, which does not treat either test as perfect, also estimated moderate sensitivity for both IgM-ELISA (0.67) and PCR (0.75) with high specificity [53]. Neither test alone is therefore a reliable gold standard.

The large regional difference in PCR positivity (3.8% in Iran vs 29.1% in Arab-country studies) deserves caution. It may reflect genuine differences in incidence, but the Arab-country studies were few, smaller, more often reconstructed from percentages, and in some cases published in journals with quality concerns. The drop in PCR positivity to 4.8% when studies with venue concerns or internal inconsistencies were excluded points the same way. The pooled PCR positivity of 7.7% is close to the 10% reported in an earlier meta-analysis of PCR in spontaneous abortion [8].

4.3 Implications for practice

For investigating spontaneous abortion, our findings support a stepwise approach. IgM-ELISA on maternal serum is a reasonable first-line test: across the cross-tabulated cohorts, most IgM-positive women also had parasite DNA detected (pooled PPV 88%, ranging from 67% to 100% and depending on prevalence), and a positive result should be followed by IgG avidity to date the infection [15, 16]. A negative IgM does not exclude abortion-associated infection, so PCR on maternal blood and/or placental or abortion tissue should be considered, ideally with a multicopy target such as RE-529 and with rigorous contamination controls [18, 19]. IgG-ELISA alone should be reported as evidence of past exposure, not as a cause of the abortion. The same reasoning applies to serosurveys that use IgG positivity in women with miscarriage as evidence of causation.

4.4 Strengths and limitations

To our knowledge, this is the first meta-analysis to quantify the within-patient agreement between ELISA and PCR for T. gondii in spontaneous abortion. We used exact-likelihood random-effects models suited to rare events, reported prediction intervals, and tested the robustness of the main positivity estimates in several sensitivity analyses.

The limitations are substantial. The evidence is concentrated in Iran and a few Arab countries, so it may not generalise to Europe, the Americas, Africa or Asia. Only eight studies provided 2 × 2 tables (seven for IgM, six for IgG), and the accuracy estimates are imprecise. PCR is not a perfect reference standard, so "sensitivity" and "specificity" here measure agreement with PCR rather than true accuracy, and differential misclassification is likely. Several studies were available only as abstracts, and some counts were reconstructed from percentages. Specimens, PCR targets, formats and ELISA kits varied, and timing of sampling relative to the abortion was rarely reported. The search depended on web-accessible databases, and title-level screening counts were not recorded, so studies in local or non-indexed journals may have been missed. Finally, the review was not registered.

4.5 Research implications

A prospective, multicentre diagnostic study is needed in which consecutive women with spontaneous abortion undergo IgM and IgG ELISA with avidity, together with standardised RE-529 real-time PCR on both maternal blood and placental tissue. Results should be analysed with latent-class methods and reported following STARD, with full 2 × 2 tables and exact timing of sampling.

5. CONCLUSIONS

In women with spontaneous abortion, IgM-ELISA and PCR detect T. gondii in a similar proportion of cases and agree substantially, whereas IgG-ELISA reflects mostly past exposure and agrees with PCR no better than chance. Neither IgM nor PCR alone identifies every infected case. Combining maternal IgM (with avidity) with PCR appears the most defensible diagnostic strategy, although this combination has not been evaluated directly, and IgG positivity alone should not be used to attribute an abortion to toxoplasmosis. Better-designed, multicentre diagnostic studies are required to confirm these conclusions.

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