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Exatidão do NIPT na deteção de T21,T18 e T13 em gestações gemelares: Revisão sistemática / Accuracy of NIPT for detection of T21,T18 and T13 in twin pregnancies: Systematic review

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Ciências médicas da saúde

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Accuracy of NIPT for detection of T21,T18 and T13 in twin pregnancies: Systematic review

ORIENTADOR

Alexandra Matias Pereira da Cunha Coelho de Macedo

COORIENTADOR (se aplicável)

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Dedicatória

Dedico este trabalho, à minha mãe , Maria da Paz, por acreditar sempre em mim.

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NIPT for detection of T21,T18 and T13 in twins

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Accuracy of non-invasive prenatal test (NIPT) for detection of trisomy 21, 18 and 13 in twin pregnancies: a systematic review

Abstract

The aim of this systematic review was to evaluate the performance of non-invasive prenatal test (NIPT) in recognising trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) in twin pregnancies. Web of Science, Medline and ClinicalTrials databases were searched electronically from January of 2020 up to 24th of December 2020. Studies were selected based on eligibility criteria and availability regarding outcome data. Out of 280 identified titles arising from the search strategy, 11 studies were included. Of these, four corresponded to twin pregnancies and seven concerned singleton pregnancies. In the primary target group, detection rate as well as specificity was outstanding reaching values of 100% regarding T21. The equivalent cannot be assumed for T18 and T13 due to the reduced number of studies reporting accuracy. The performance of NIPT for all aneuploidies in singletons was up to the mark and as predicted from prior studies.

Keywords: aneuploidy; cell-free DNA; detection rate; non-invasive prenatal test; prenatal diagnosis; sensitivity and specificity

What is already known?

The robustness of the test in singleton is widely recognized. In multiple pregnancies, lack of sufficient data and challenging problems derived from the fluctuation of cell-free DNA and its origin regarding each fetus in dizygotic twins can be puzzling. Scarce studies are available ascertaining NIPT clinical performance in twin pregnancies.

What the results of this study add?

There is no coherent evidence on the outcome of NIPT screening performance in aneuploidies regarding twin pregnancies. Furthermore, the reliability of this method concerning each trisomy (T21, T18 and T13) is not clear. NIPT in twin pregnancies demonstrated an equal capacity of screening for T21 as for singletons, whereas regarding T18 and T13 the test was less performant.

What are the implications of these findings for clinical practice and further research?

The goal of this study was to assess the exactness of aneuploidy screening in multiple pregnancies. Secondary outcome was to assess whether the clinical performance of the test in twins was equivalent or inferior when compared to singleton pregnancies.

Resumo

O objetivo desta revisão sistemática foi avaliar o desempenho dos testes pré-natais não invasivos (NIPT) na deteção de três trissomias (T) - T21, T18 e T13 – em gravidezes gemelares.

A pesquisa eletrónica de artigos nas bases de dados ocorreu entre Janeiro de 2020 e 24 de Dezembro do mesmo ano, tendo sido efetuada na Web of Science, Medline e Clinical Trials. O *outcome* primário consistiu na capacidade de diagnosticar aneuploidias em gravidezes múltiplas. Por sua vez, o *outcome* secundário foi comparar o poder de diagnóstico de trissomias dos NIPT em gravidezes múltiplas e únicas, tentando estabelecer uma relação de superioridade ou inferioridade. Os estudos foram incluídos com base nos critérios de elegibilidade e disponibilidade mediante o *outcome* primário supracitado.

Em 280 estudos oriundos da pesquisa efetuada, 11 foram incluídos. Destes, 4 referiam-se a gravidezes gemelares e 7 a gravidezes únicas. Nas primeiras, a especificidade e a capacidade de deteção de T21 dos NIPT foram excelentes. Em contraste, o mesmo não se poderá afirmar na deteção de T18 e T13, devido à falta de informação referente ao poder diagnóstico dos testes nestas trissomias. Como demonstrado em estudos anteriores, a boa *performance* dos NIPT em gravidezes únicas foi assinalável.

Com efeito, nas T21, os NIPT demonstraram uma capacidade de diagnóstico equivalente entre as gravidezes múltiplas e únicas. No entanto, para a deteção de T18 e T13 em gravidezes gemelares, os testes revelaram menos eficácia. Desta forma, para uma clarificação da assertividade dos NIPT em detetar T18 e T13 nas gravidezes múltiplas, será necessário mais estudos sobre o tema.

Palavras-chave**:** aneuploidia; DNA livre; capacidade de deteção; testes pré-natais não invasivos; sensibilidade e especificidade;

Introduction

Non-invasive prenatal testing (NIPT) uses cell-free foetal DNA (cfDNA) extracted from maternal blood to screen for the most common fetal chromosomal aneuploidies (Dondorp *et al*, 2015). This method was first introduced in a clinical setting in Hong Kong in 2011, and since then has disseminated throughout the world (Allyse *et al*, 2015). In singleton pregnancies, the performance of NIPT has been proven to be successful in detecting the most frequent autosomal chromosomal anomaly in the human race (T21), as well as T18 and T13.The corresponding sensitivities are 99, 98 and 99% at a combined false positive rate (FPR) of 0.13% (Gil *et al*, 2017).

Nonetheless, the question regarding performance and clinical applicability in twin pregnancies remains unanswered. This constitutes an issue to clinicians, taking into account that multiple births represents 2-3% of births globally, with a tendency to increase (Yang *et al*, 2018).This noteworthy increase in twin deliveries can be ascribed to two major causes: pregnant women are older and there is a widespread use of assisted reproductive techniques.

Nevertheless, twin pregnancies may carry a higher risk of aneuploidy than singletons, depending on the type of placentation (chorionicity) and fetal fraction (FF). For instance, in monozygotic twins both fetuses are either affected or unaffected with aneuploidy. On the other hand, in dizygotic pregnancies the risk of aneuploidy is generally independent for each fetus (Audibert and Gagnon 2017).

Available studies concerning NIPT in twin pregnancies are scarce. Both obstetricians and geneticists have called for more research in this matter, due to lack of quality and size of data (American College of Obstetricians and Gynecologists 2016). Although a previous and recent review was published, there were several limitations

regarding it. The performance of NIPT concerning T18 and T13 was unsatisfactory because of publication bias and a reduced number of cases (He *et al*, 2020).

The aim of this study is to conduct a systematic review of all available data, referring to the performance of NIPT for the detection of T21, T18 and T13 in twin pregnancies, whilst assessing the screening test accuracy with standard diagnostic methods such as amniocentesis and chorionic villus sampling.

Methods

Protocol, selection of studies and data collection

This systematic review was developed according to PRISMA guidelines (Prisma 2020) along with a new protocol following PRISMA-P recommendations, which were both registered in PROSPERO database (CRD42021231732), before its elaboration.

Studies were identified by searching electronic databases with no language restriction. Limitation of year of publication was applied pertaining studies from 2010 since NIPT was only introduced in clinical practice in 2011. This search was applied to Web of Science (2010-2020), Medline (2010-2020) and Clinical Trials. All electronic databases were run until 24/12/2020. The search strategy used a combination of medical subject heading (MeSH) , search terms and variants for NIPT test , trisomies and accuracy such as: “NIFTY”, “ prenatal diagnosis” “non-invasive prenatal”, “ cell-free DNA” , “ Harmony”, “ Bambni Test” , “Panorama”, “chromosomal”, “trisomy”, “ aneuploidy”, “Down”, “Edwards” , “ Patau”, “Sensitivity and Specificity” or “ diagnose”, among others that can be fully seen in the provided appendix. Abstracts and titles were reviewed independently by two authors (TC, VS). Disagreements were solved by consensus or by a third author (AM). Inclusion criteria sought out articles which explored the performance of NIPT in pregnant women (twins and singletons

separately). The standard reference was invasive diagnostic procedures (amniocentesis, chorionic villus sampling, cordocentesis) or conventional screening. The primary outcome was any clinical applicability (sensitivity, specificity, positive predictive value, negative predictive value, false-positive rate, false-negative rate) of NIPT in detecting T21,T18 and T13 in twin pregnancies. Secondary outcome aimed to answer if the performance of NIPT in twins was equal or inferior to NIPT in singletons. Solely the studies with reported outcomes on diagnostic accuracy were included in this review. Letters, reviews, editorials and communications containing insufficient information on methods and no numerical outcomes data were excluded. For the articles with no results posted the respective authors were contacted. The same two authors extracted independently the pertinent data from the full text included studies. Any discrepancies were discussed between them and consensus was reached through this or by the intervention of the third reviewer. Data eluded from each study included: type of population (singletons or twins separately), classification of the intervention (Next-generation sequencing, Massive parallel sequencing, Single-nucleotide polymorphism), form of control and display of outcome measurements regarding the intervention (sensitivity, specificity, positive predictive value, negative predictive value, false-positive rate, false-negative rate).

Quality assessment, risk of bias and statistical analysis

The quality of the included studies was performed using a modified Quality Assessment Tool for Diagnostic Accuracy Studies-2 checklist (QUADAS-2) (Whiting *et al*, 2011). As stated in QUADAS-2 each study is based on four domains: patient selection, index test, reference standard, flow and timing. The prime domain was considered as low risk of bias if patients were enrolled in a consecutive or random manner and refrained from improper exclusions. In regard to the second domain, studies were viewed as low risk

provided the results of the index test had been interpreted without prior knowledge of the gold standard. The reference standard was invasive diagnostic tests and conventional screening methods. However, included studies used solely karyotyping as source. The gold-standard was perceived as low risk assuming it was likely to identify the targeted condition. The last domain refers to flow and timing. Articles were deemed as minor risk if: the index test was performed before the reference standard and whether all patients received the reference standard. Applicability of studies was assessed based upon inclusion criteria.

Two modifications were made. First, an additional signalling question was added to determine if the index test was performed before the reference standard. Thus, we could determine whether or not the diagnostic accuracy of NIPT was being enhanced by the increase of foetal fraction following the invasive procedure. Secondarily, the standard signalling question on whether there was an appropriate interval between the index test and reference standard was eliminated, since it would not have any impact on accuracy.

In order to avoid publication bias the review followed a-priori protocol which was registered with fully transparency in POSPERO and QUADAS-2 was performed in all included studies.

From systematic review procedures, it is possible to perform a statistical combination of results, i.e., a meta-analysis. Meta-analyses allow researchers to pool average effect estimates from different studies, test the robustness of results across studies with high precision, and make sense of apparently conflicting study results or establish new discoveries in the research field. The use of a meta-analytic procedure in this work would provide information on how well a certain test classifies trisomy (T18, T13, T21) during pregnancy. However, meta-analyses also have the potential to mislead

seriously and be biased, particularly if there are few studies in the field and higher variation across studies. The precision of estimates in a meta-analysis typically increases with the quantity of data (Macaskill *et al,* 2010, Higgins *et al*, 2019). As data increases, researchers will have more power to quantify differences in test accuracy, to calculate more precise estimates of expected sensitivity and specificity, to describe which differences are likely to be real (or, alternatively, are explained by change) and which differences can be explained by known differences in study characteristics.

In this review, few studies were suitable to meta-analytic procedures since articles not reporting specificity or sensitivity across tests (T13, T18, T21) and groups (twins, singletons) could not enter in the quantitative analysis. The number of studies included in the current work ranged from one (T13, singleton group) to six (T21, twin group). This limited the use of a meta-analytic approach in the current work and, specifically, the calculation of general effect sizes, heterogeneity indicators, and publication bias. Alternatively, we calculated a pooled mean for each trisomy test, sensitivity/specificity indexes, and pregnancy groups to provide a combined estimate of the weighted average of sensitivity and specificity effects across these variables, while discussing study characteristics and their possible effects for the interpretation of the results.

Results

Study selection, characteristics

Two hundred and eighty records were identified after duplicates were removed, of which 32 were assessed for their eligibility regarding inclusion criteria. Eleven studies were incorporated in this systematic review (Tables 1 and 2). Figure 1 summarises this process. Four studies included uniquely twin pregnancies, whereas seven studies encompassed only singleton pregnancies (Bianchi *et al*, 2012, Grömminger *et al*, 2014, Hall *et al*. 2014, Huang *et al*, 2014, Zhou *et al*, 2014, Alberti *et al*, 2015, Zhang and Zhang 2016, Malan *et al*, 2018, Kypri*et al*, 2019, Norwitz*et al*, 2019, Rousseau *et al*, 2019).

All studies regarding twin pregnancies where cohort (n=4), whereas articles concerning singleton pregnancies constituted a combination of case-control (n=2), randomised control trial (n=2) and cohort (n=2) designs. The preponderance of studies tested diagnostic accuracy in a high-risk population (n=5), mixed-risk population (n= 4) and one study did not specify the type of population. The results of quality assessment using QUADAS-2 are presented in Figure 2 and 3. Most of the included studies demonstrated an overall low risk of bias throughout the four domains. Two of the four domains were associated with a higher risk of bias: patient selection and study flow. Regarding patient selection: one study presented with high risk of bias, whereas two others were deemed as unclear. Flow and timing were viewed as unclear in one study and considered high risk in three. As for the reference standard along with the index test, risk of bias was regarded as low.

Discussion

Main findings

In a systematic review of 432 studies, we were able to discover 11 eligible articles with reference to NIPT accuracy in detecting T21, T18 and T13 in two specific types of population: twins and singletons. Quality assessment employed by QUADAS-2 suggested an overall low risk of bias in the majority of included studies. Even though there was a slight apprehension about unclear risk of bias regarding two domains, this was due to equivocal statements and possibly obliterated information. However, applicability was not viewed as a concern.

The results from this review as to outcome analysis demonstrated a pooled mean sensitivity of 100% for T21 in twin pregnancies throughout all included studies (Figure 4). As for T18 the pooled mean detection rate obtained was 84.406%, with a single study reporting a true positive rate of 50%, whereas the other 3 articles maintained the global detection rate at 100%. Apropos of T13, NIPT demonstrated a pooled mean sensitivity of 100% in one study. However, these last results should be carefully interpreted since the number of included studies which investigated T13 in twin pregnancies were scarce (n=1) and should not lead to misleading generalisation. Every study in reference to multiple pregnancies revealed a pooled mean specificity of 100% in all three conditions (T21,T18 and T13). On the other hand, outcomes measures derived of singleton pregnancies did not differ considerably from those found in twins (Figure 5). Of the seven studies incorporated, NIPT capacity in detecting T21 maintained the sensitivity mean about 99,746% along with a specificity of 99,642%. In addition, NIPT demonstrated a pooled mean detection rate of 99,486% and a true negative rate of 99,639% regarding T18. Lastly, the test screening characteristics

concerning T13 revealed a sensitivity of 98,042% associated with a specificity of 99,638%.

Limitations and strengths

Exhaustive search approach was carried out in this review, in order to recognise all available data pertaining NIPT and its clinical performance in identifying T21,T18 and T13 in twin pregnancies as in singleton. Nonetheless, there were some limitations, which restrained this review in such a complex matter as this one. The small number of cases in the studies, the non-randomised design as well as missing information from variables deemed of paramount significance such as placentation, zygosity, fetus affected, fetal fraction (FF%), and maternal age constituted considerable hinderance.

Clarification of findings and relation to other published studies

The conclusions reached by this review corroborate the findings of previous studies stating that NIPT has a high clinical performance in twin pregnancies (Takeda *et al*, 2018). Primary results seem to indicate that the detection of T21 by NIPT in twins is tantamount to its performance in singletons, though further as sizeable studies are called for. As for T18 as well as T13, lack of data is still an issue to deduce identical conclusions. One could defend the view that NIPT is not acceptable for multiple pregnancies due to the difficulties inherent to this population: which fetus is affected and which fetal fraction contributed from each one, placenta mosaicism, vanishing twin, among other reasons. However, one cannot argue against the low false negative rate and elevated specificity the test has to offer for anxious couples. Therefore, this test has the capacity of avoiding unnecessary invasive procedures. Promising results have been demonstrated and little doubt remains about NIPT performance, in comparison with other non-invasive screening methods.

Clinical and research inferences

Integration of NIPT in clinical practice may revolutionise obstetrical clinical practice. There have been many studies in several countries investigating NIPT feasibility and implementation. One study performing a 2 year experience regarding NIPT reported a reduced number of invasive diagnostics as procedure-related risks (Noh*et al*, 2019). A different article delivered a pioneering large-scale clinical investigation of NIPT as first-tier test using Dongguan, China as an experimental city. The results were more than reassuring since they showed that NIPT had a lower false-positive rate that conventional serum screening (Liu *et al*, 2020). A current study showed in their paper that NIPT achieved greater results than first-trimester combined test or second-trimester biochemical test along with no false negative results (Motevasselian *et al,* 2020). One specific work pinpoint the principal rationale for NIPT screening failure being: low fetal fraction, elevated maternal body mass index, vanishing twin, cancer along with others (Luo *et al,* 2020). Furthermore, a particular research was able to enhance the positive predictive value (PPV) of the test by establishing a second-generation NIPT along with scientific modifications in order to diminish false-positive results (Strom *et al,* 2017). Recent investigation have proven that to determine zygosity is one of the major difficulties in this matter, taking into account the approaches used for monozygotic and dizygotic differ from one another. A distinct study demonstrated that the performance in singletons and twins is equal and can be carefully provided to women bearing multiple fetuses, especially since the risk of invasive diagnostic procedures is much higher in twins. In addition, it states that cell-free DNA-based NIPT is the leading screening method for common aneuploidies so far (Verde *et al*, 2021). However, some more research has to be done until NIPT can be offered as routine screening in twins. Additional studies regarding accuracy of NIPT, implementation of the test in a clinical

setting and cost-effectiveness are needed for further evaluation.

Conclusion

Accuracy of NIPT in twin pregnancies will always be more challenging than in singletons.This systematic review was able to corroborate useful clinical performance for the detection of T21, whereas for T18 and T13 it was unable to elucidate with reliable strength. Furthermore, a greater amount of studies on this matter is necessary for establishing clinical performance in multiple pregnancies and to strengthen its implementation in routine prenatal care.

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Declaration of interest

The authors report no conflicts of interest.

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Table 1- General characteristics of the included studies regarding twin pregnancies

Author	Study design	Country and year	Condition	Pregnancies with trissomy (TP) / Pregnancies (n)	Placentation of pregnancies (n) / Placentation of affected pregnancies	NIPT result	Outcomes	FF	Karyotype	Fetuses affected	MA	GA	NIPT method	Zygosity	Type of population
Huang, Xuan, et al	Cohort	China- 2014	T21 and T18	11/189	31/ 1 MCDA 2/ NS MCMA 152 / 7 DCDA	9 T21 1 T18	T21 Sens=100% Spec=100% T18 Sens= 50% Spec= 100%	NS	9 T21 2 T18	1 in all 11 positive cases	Range=22-44years Median= 31	Range=11-36weeks Median= 19weeks	MPS[s]	NS	High-risk
Grömminger, Sebastian, et al	Cohort	Germany-2014	T21, T18 and T13	**R:** 4/16 **P:** 2/38	**R -**NS **P:** 23 / 1 DCDA 5/1 MC (NS amniocity) 3/0 MCDA 1/0 MCMA	**R:** 4 T21 **P:** 2 T21	**R:** T21 Sens=100% Spec=100%	**R**: 8-45% **P:** 5.4-24.8%	**R**: 4 T21 **P:** 2 T21	**R:** 1 in all 4 cases **P:** 2 fetuses in MC case; 1 fetus in DCDA case	NS	Range= 10 + 6; 18 + 4 weeks + days	rMPS[e]	NS	NS
Kypri, Elena, et al	Cohort	USA-2019	T21, T18 and T13	5/300	NS	3 T21 1 T18 1 T13	T21 Sens=100% Spec=100% T18 Sens= 100% Spec= 100%	NS	NS	NS	NS	>10weeks	TACS[f]	NS	Mixed-risk
Norwitz, Errol R, et al	Cohort	USA-2019	T21,T18 and T13	11/117	NS	5 T21 5 T18 1 T13	DZ[a] : T21 Sens=100% Spec=100% T18 Sens= 100% Spec= 100% T13 Sens=100% Spec=100%	NS	5 T21 5 T18 1 T13	NS	Mean ± SD= 33.0 ± 5.5 years	Mean ± SD=15.6 ± 4.8weeks	SNP[g]	**All cases** 40/1 MZ 77/6 DZ **Affected cases** 5 T21 - 4 DZ & 1MZ 5 T18 - DZ 1 T13 - DZ	Mixed-risk

TP= true positives; FF= Fetal fraction; MA= Maternal age; GA= Gestacional age; NS= not specified; DCDA= dichorionic diamniotic; MCDA= monochorionic diamniotic; MPS= Massively parallel sequencing; rMPS= Random Massively parallel sequencing; TACS= Custom target capture sequences; SNP= Single-Nucleotide Polymorphism; MZ= Monozygotic; DZ= Dizygotic; Sens= Sensibility; Spec=Specificity; R= Retrospective part ; P= Prospective part; [a]=MZ aneuploidy sensitivity was not determined due to insufficient sample numbers (n = 1).

Table 2. General characteristics of the included studies regarding singleton pregnancies.

Author or study	Study design	Country and year	Condition	Pregnancies with trissomy (TP)/ Pregnancies (n)	NIPT result	Outcomes	Karyotype	MA	GA	Population	FF	NIPT method
Bianchi, Diana W., et al	Nested case-control	USA-2012	T21,T18 and T13	89/493 T21 36/496 T18 14/499 T13	89 T21 35 T18 11 T13	T21 Sens=100% (95.9–100.0) Spec=99.9% (99.1–100.0) T18 Sens= 97.2% (85.5–99.9) Spec= 100% (99.2–100.0) T13 Sens=78.6%(49.2–99.9) Spec= 100% (99.2–100.0)	89 T21 36 T18 14 T13	Mean± SD(35.2±6.40) years. Range: 18–46years	Mean± SD(15.1±3.16) weeks Range: 10–23 weeks	High-risk pregnancies	NS	MPS
Hall, Megan P., et al	Case- control	USA- 2014	T13	15/64	15 T13	T13 Sens=100%(78.2–100%) Spec=100% (92.8–100%)	15 T13	> 35y	Median= 16 weeks (12.1–22.7 weeks).	High-risk pregnancies	Mean:12.1% Median: 11.1%, Range: 2.2–30.4%	SNP
Zhou, Qiyin, et al	Cohort	China- 2014	T21,T18 and T13	48/3948	38 T21 12 T18 4 T13	T21 Sens=100% Spec=99.9 (99.9–100%) FPR=0.05 (0.02–0.10%) PPV=94.7 (94.2–95.2%) T18 Sens= 100% Spec= 99.9 (99.9–100%) FPR=0.05 (0.02–0.10%) PPV= 83.3 (82.9–83.7%) T13 Sens=100% Spec=99.9 (99.9–100%) FPR=0.05 (0.02–0.10%) PPV=50.0 (48.9–51.1%)	36 T21 10 T18 2 T13	>35y and <35y with high risk for Down syndrome	Range: 12-24 weeks	Mixed population	NS	MPS
Alberti, A., et al.	Case-control	France-2015	T21	47/183	47 T21	T21 Sens=100% (94.1–100.) Spec=100% (98.–100.)	47 T21	Mean± SD (35.2 ± 6.7)	Mean± SD (14 ± 2)	High-risk pregnancies	NS	MPS
Zhang, Jiao, and Bin Zhang	Cohort	China-2016	T21 and T18	4/ 87	3 T21 1 T18	T21 Sens=100% Spec=100% FPR=100% PPV=100% T18 Sens=100% Spec=100% FPR=100% PPV=100%	3 T21 1 T18	Mean± SD (37.48±2.17 years)	Median: 19.0 weeks (12.4-32.5 weeks).	High-risk pregnancies	NS	NGS

Malan, Valérie, et al.	RCT	France-2018	T21	27/984	82 T21-27 TP and 55 FP	T21 Sens=100%(87.2%-100%) FPR= 5.6%(4.2%-7.2%)	27 T21	Mean± SD (36.2 ±5.2 years)	Mean± SD(15.0± 1.3)	High-risk pregnancies	NS	NGS
Rousseau, François, et al	RCT	Canada-2019	T21,T18 and T13	Proton™ 199/1642 HiSeq™ 202/1650	Proton™ 6 T13, 48 T18 and 150 T21 HiSeq™ 7 T13, 49 T18 and 151 T21	T21, T18 and T13 (both tests HiSeq™; Proton™) Sens= > 99% (96—100%) Spec= 99% (98—100%) NPV = ≥99.8% (99—100%) A combined FPR (T13, T18, or T21)= 0.9% (0.5—2%)	Proton™ 6 T13, 48 T18 and 152 T21 HiSeq™ 7 T13, 49 T18 and 152 T21	Mean± SD(34 ± 5.4)	Mean± SD(17.1± 3.2)	Mixed population	Range: 4–35%	NGS

NS= not specified; MPS= Massively parallel sequencing; rMPS= Random Massively parallel sequencing; TACS= Custom target capture sequences; SNP= Single-Nucleotide Polymorphism; Sens= Sensibility; Spec=Specificity.

Figure captions

Figure 1. PRISMA flow diagram.

Figure 2. Proportion of studies with low, high or unclear risk of bias using QUADAS 2.

Figure 3. Proportion of studies with low, high and unclear concerns regarding applicability using QUADAS 2.

Figure 4. Pooled mean concerning twin pregnancies.

Figure 5. Pooled mean concerning singleton pregnancies.

Figure 1

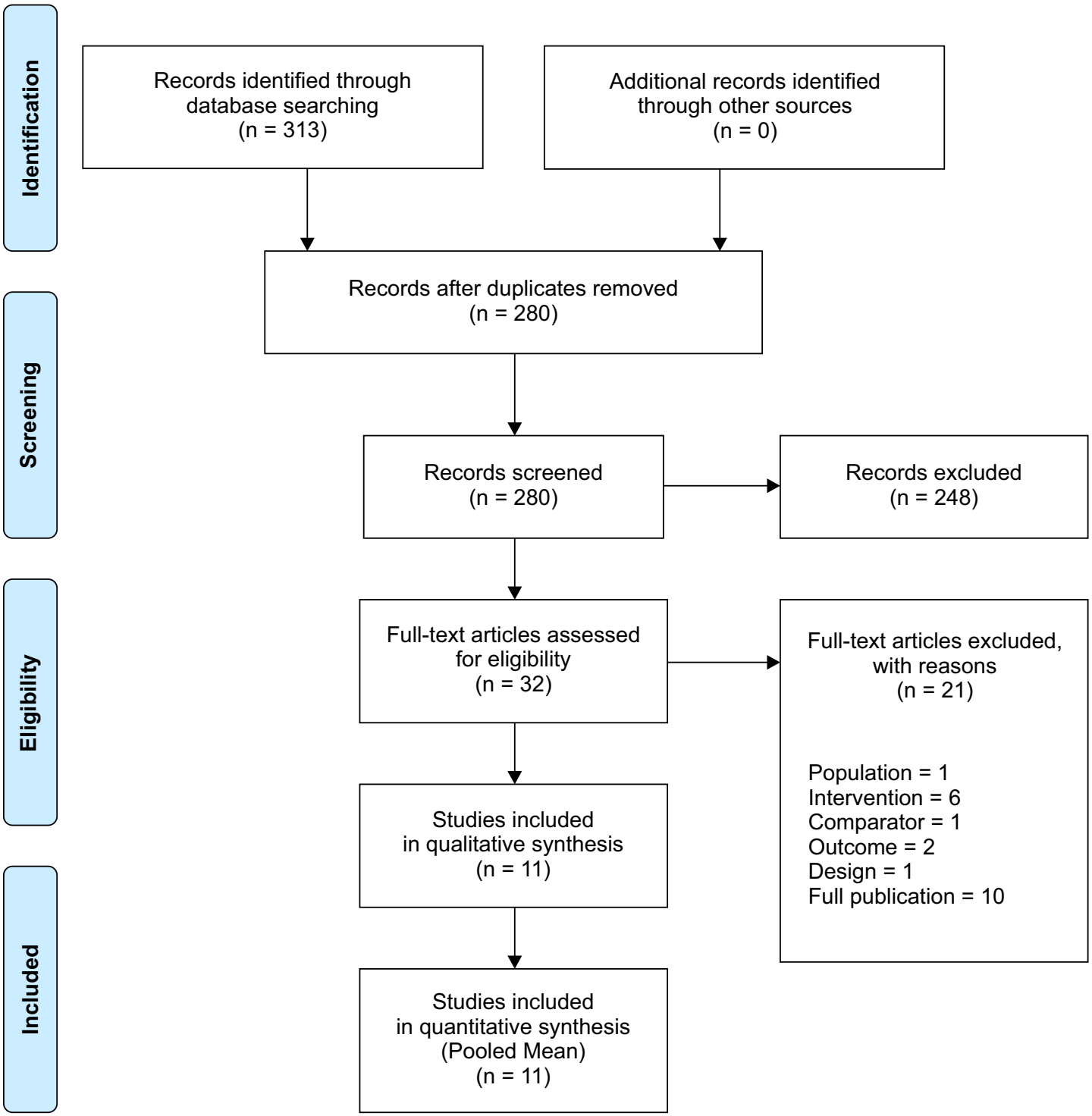
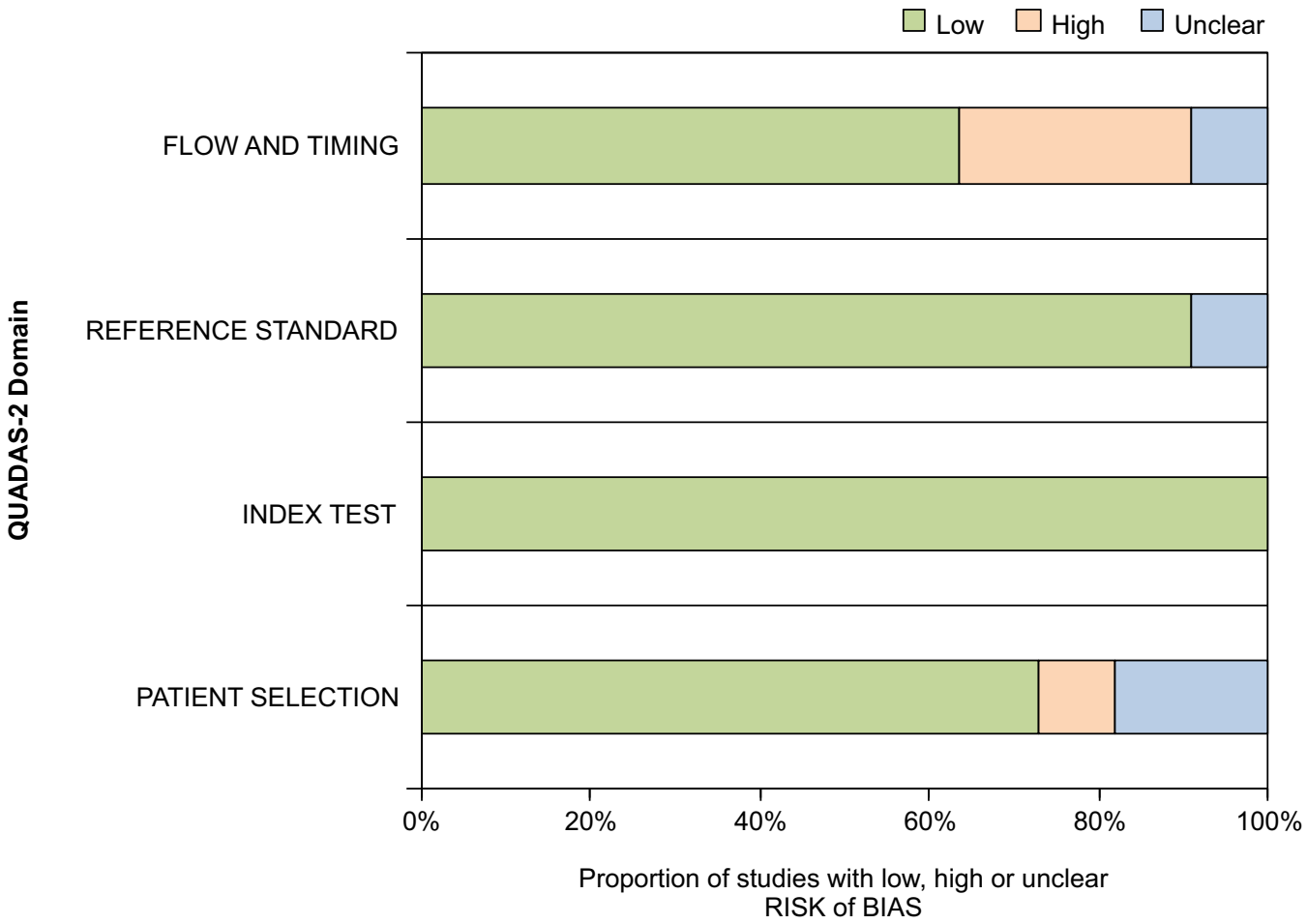

Identification
Screening
Eligibility
Included
Records identified through database searching (n = 313)
Additional records identified through other sources (n = 0)
Records after duplicates removed (n = 280)
Records screened (n = 280)
Records excluded (n = 248)
Full-text articles assessed for eligibility (n = 32)
Full-text articles excluded, with reasons (n = 21)
Population = 1
Intervention = 6
Comparator = 1
Outcome = 2
Design = 1
Full publication = 10
Studies included in qualitative synthesis (n = 11)
Studies included in quantitative synthesis (Pooled Mean) (n = 11)

Figure 2 and 3



.

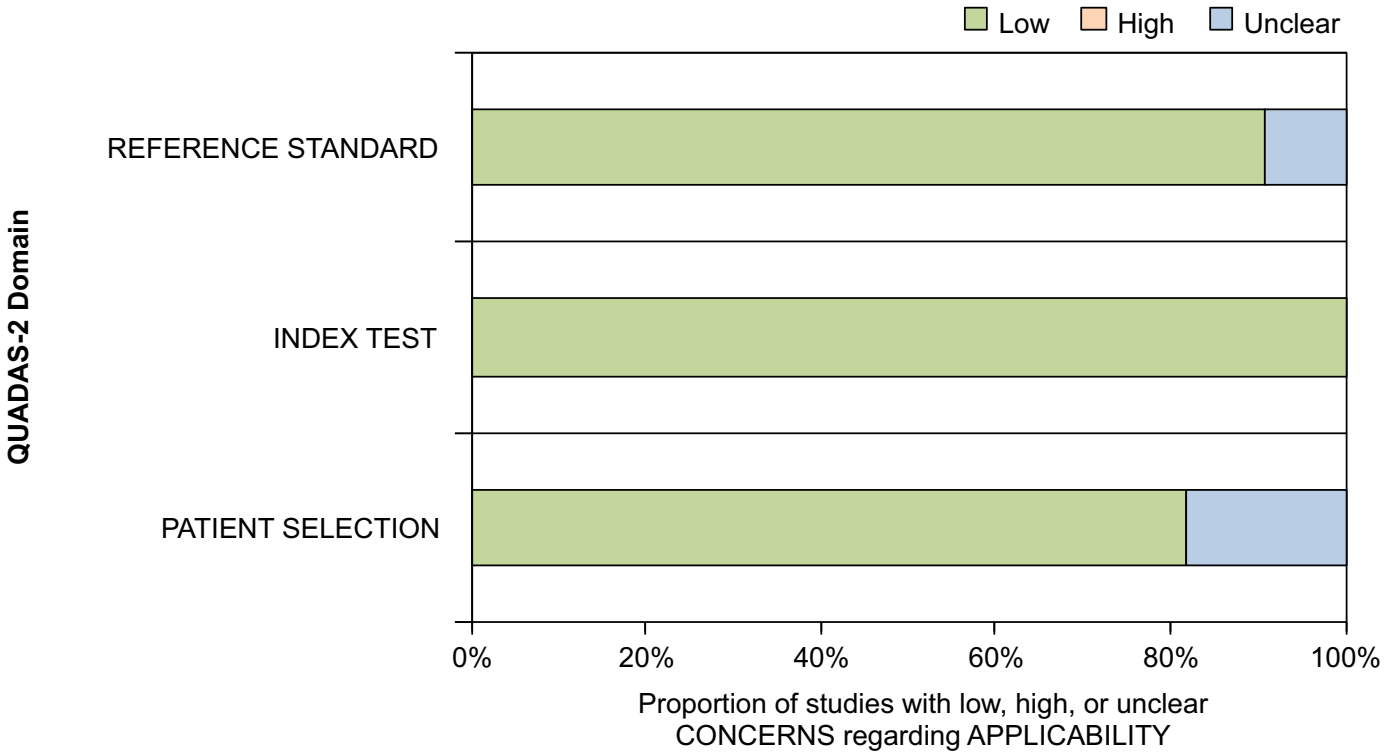
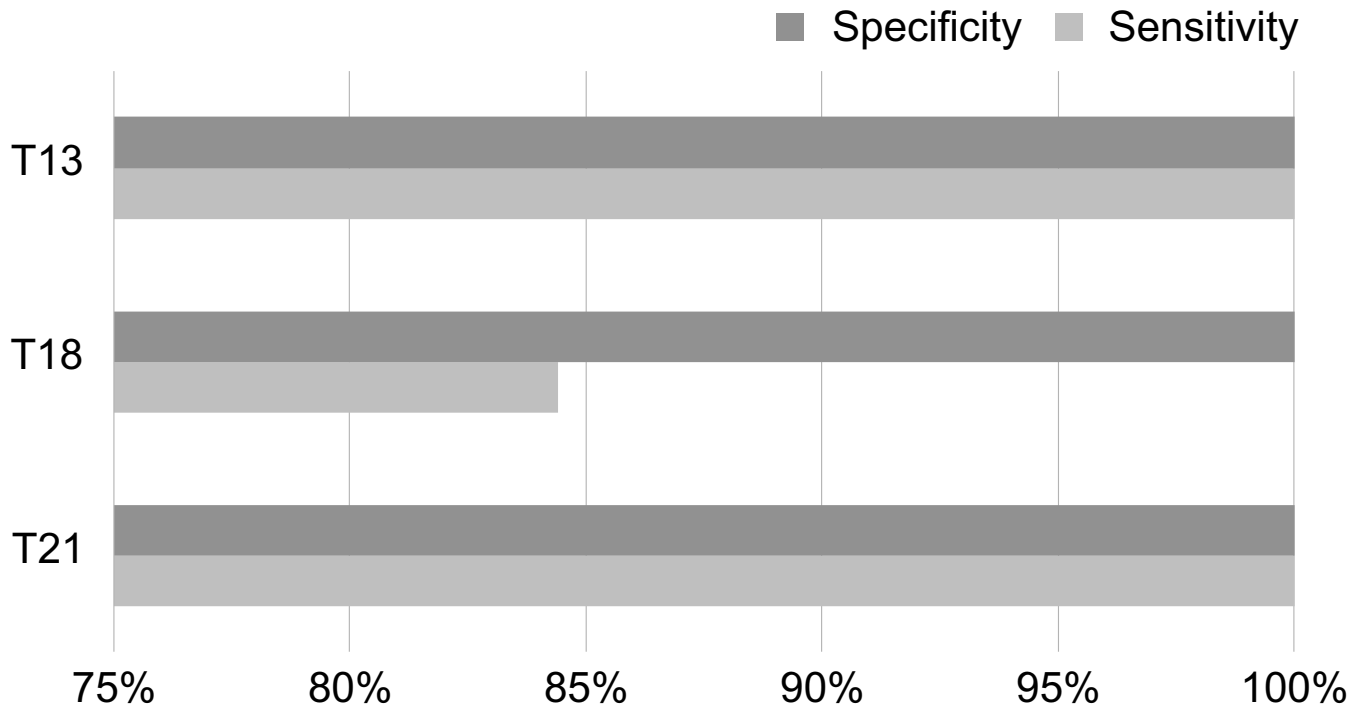
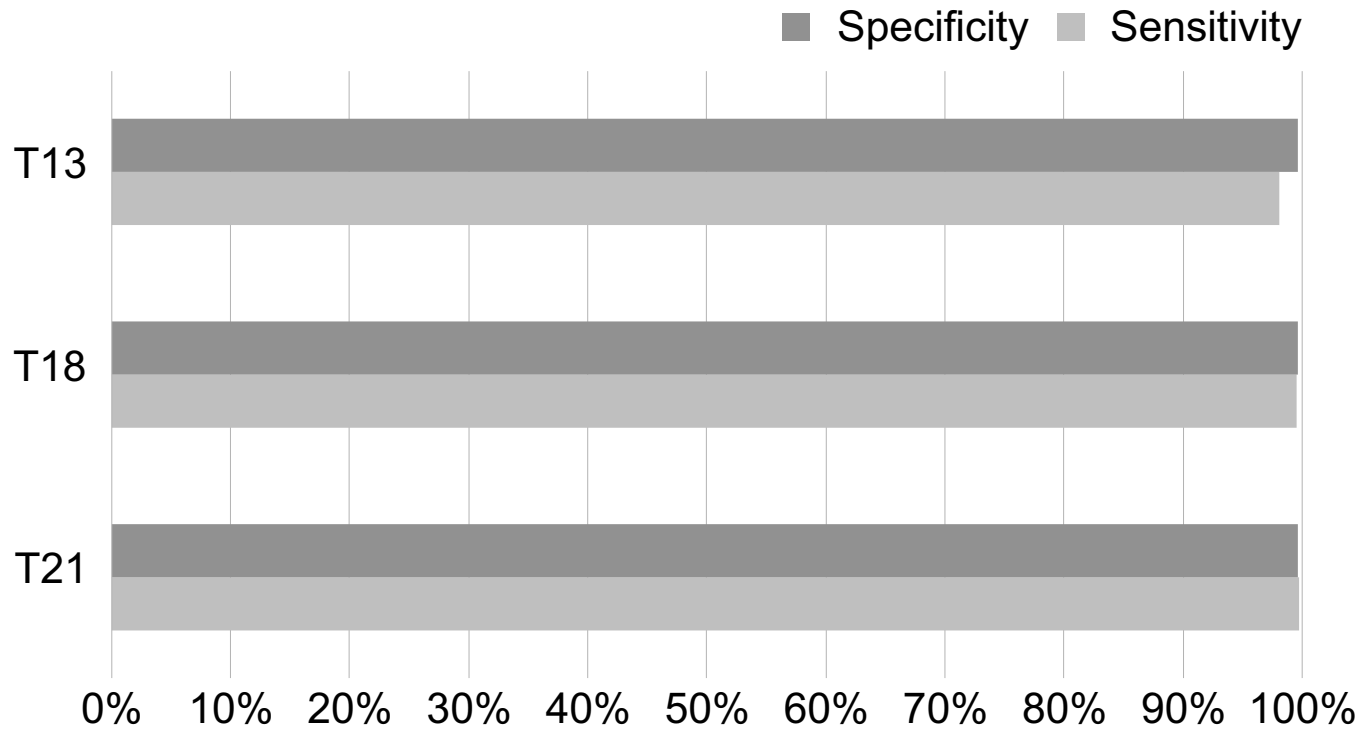


Figure 4 and 5





ANNEX 1

Journal of obstetrics and Gynaecology (Q3- indexed to Web of Science)

Instructions for authors

About the Journal

Journal of Obstetrics and Gynaecology is an international, peer-reviewed journal publishing high-quality, original research. Please see the journal's Aims & Scope for information about its focus and peer-review policy.

Please note that this journal only publishes manuscripts in English.

Preparing Your Paper

All authors submitting to medicine, biomedicine, health sciences, allied and public health journals should conform to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, prepared by the International Committee of Medical Journal Editors (ICMJE).

Structure

Your paper should be compiled in the following order: title page; abstract; keywords; main text introduction, materials and methods, results, discussion; acknowledgments; declaration of interest statement; references; appendices (as appropriate); table(s) with caption(s) (on individual pages); figures; figure captions (as a list).

Title pages for all article types need to have the full title included, all authors' names and affiliations and the name and contact information for one corresponding author.

Title pages for Original Articles should have a running head (a shortened version of the full title, which can be up to 10 words in length).

Word Limits

Please include a word count for your paper.

Original Article: Maximum 3000 words *Original laboratory or clinical contributions

Review Article: Maximum 5000 words

Case Report: Maximum 800 words

Short communication: Maximum 800 words, *1 figure or table and 10 references, at the Editor's discretion

Correspondence: Maximum 400 words

Book reviews: Maximum 5000 words

* The word counts do not include the Reference list, Tables and Figures.

Style Guidelines

Please refer to these quick style guidelines when preparing your paper, rather than any published articles or a sample copy.

Please use British (-ise) spelling style consistently throughout your manuscript.

Please use single quotation marks, except where ‘a quotation is “within” a quotation’. Please note that long quotations should be indented without quotation marks.

In writing your paper, you are encouraged to review articles in the area you are addressing that have been previously published in the Journal, and where you feel appropriate, to reference them. This will enhance context, coherence, and continuity for our readers. Authors should include telephone numbers as well as e-mail addresses on the cover page of manuscripts. The use of footnotes is not permitted. For original papers the abstract should also include a separate impact statement (max 200 words). This should include the following information: - What is already known on this subject. - What the results of this study add. - What the implications are of these findings for clinical practice and/or further research.

Formatting and Templates

Papers may be submitted in Word format. Figures should be saved separately from the text. To assist you in preparing your paper, we provide formatting template(s).

Word templates are available for this journal. Please save the template to your hard drive, ready for use.

If you are not able to use the template via the links (or if you have any other template queries) please contact us here

References

Please use this reference guide when preparing your paper.

Checklist: What to Include

1. **Author details.** Please ensure everyone meeting the International Committee of Medical Journal Editors (ICJME) requirements for authorship is included as an author of your paper. All authors of a manuscript should include their full name and affiliation on the cover page of the manuscript. Where available, please also include ORCiDs and social media handles (Facebook, Twitter or LinkedIn). One author will need to be identified as the corresponding author, with their email address normally displayed in the article PDF (depending on the journal) and the online article. Authors' affiliations are the affiliations where the research was conducted. If any of the named co-authors moves affiliation during the peer-review process, the new affiliation can be given as a footnote. Please note that no changes to affiliation can be made after your paper is accepted. Read more on authorship.
2. Should contain an unstructured abstract of 150 words.
3. **Graphical abstract** (optional). This is an image to give readers a clear idea of the content of your article. It should be a maximum width of 525 pixels. If your image is narrower than 525 pixels, please place it on a white background 525 pixels wide to ensure the dimensions are maintained. Save the graphical abstract as a .jpg, .png, or .gif. Please do not embed it in the manuscript file but save it as a separate file, labelled GraphicalAbstract1.
4. You can opt to include a **video abstract** with your article. Find out how these can help your work reach a wider audience, and what to think about when filming.
5. Between 5 and 6 **keywords**. Read making your article more discoverable, including information on choosing a title and search engine optimization.

6. **Funding details.** Please supply all details required by your funding and grant-awarding bodies as follows:

   For single agency grants

   This work was supported by the [Funding Agency] under Grant [number xxxx].

   For multiple agency grants

   This work was supported by the [Funding Agency #1] under Grant [number xxxx]; [Funding Agency #2] under Grant [number xxxx]; and [Funding Agency #3] under Grant [number xxxx].

7. **Disclosure statement.** This is to acknowledge any financial interest or benefit that has arisen from the direct applications of your research. Further guidance on what is a conflict of interest and how to disclose it.

8. **Data availability statement.** If there is a data set associated with the paper, please provide information about where the data supporting the results or analyses presented in the paper can be found. Where applicable, this should include the hyperlink, DOI or other persistent identifier associated with the data set(s). Templates are also available to support authors.

9. **Data deposition.** If you choose to share or make the data underlying the study open, please deposit your data in a recognized data repository prior to or at the time of submission. You will be asked to provide the DOI, pre-reserved DOI, or other persistent identifier for the data set.

10. **Supplemental online material.** Supplemental material can be a video, dataset, fileset, sound file or anything which supports (and is pertinent to) your paper. We publish supplemental material online via Figshare. Find out more about supplemental material and how to submit it with your article.

11. **Figures.** Figures should be high quality (1200 dpi for line art, 600 dpi for gray-scale and 300 dpi for colour, at the correct size). Figures should be supplied in one of our preferred file formats: EPS, PS, JPEG, GIF, or Microsoft Word (DOC or DOCX). For information relating to other file types, please consult our Submission of electronic artwork document.
12. **Tables.** Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text. Please supply editable files.
13. **Equations.** If you are submitting your manuscript as a Word document, please ensure that equations are editable. More information about mathematical symbols and equations.
14. **Units.** Please use SI units (non-italicized).

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You must obtain the necessary permission to reuse third-party material in your article. The use of short extracts of text and some other types of material is usually permitted, on a limited basis, for the purposes of criticism and review without securing formal permission. If you wish to include any material in your paper for which you do not hold copyright, and which is not covered by this informal agreement, you will need to obtain written permission from the copyright owner prior to submission. More information on requesting permission to reproduce work(s) under copyright.

Disclosure Statement

Please include a disclosure statement, using the subheading "Disclosure of interest." If you have no interests to declare, please state this (suggested wording: *The authors*

report no conflict of interest). For all NIH/Wellcome-funded papers, the grant number(s) must be included in the declaration of interest statement. Read more on declaring conflicts of interest.

ANNEX 2

Section/topic	#	Checklist item	Reported on page and paragraph/ table #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both. **- MANDATÓRIO**	8 and 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. **– SEGUIR RECOMENDAÇÕES DA REVISTA**	8 and 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. **– MANDATÓRIO** ***O rationale corresponde à justificação da importância da revisão sistemática***	11 and 1- 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). **- MANDATÓRIO**	12 and 1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. **– FACULTATIVO**	12 and 2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. **– MANDATÓRIO** ***É altamente recomendado, de acordo com as boas práticas da Cochrane, que não sejam aplicados critérios de exclusão baseados na língua e/ou data de publicação dos estudos.***	12 and 3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. **– MANDATÓRIO** ***Em consonância com as boas práticas da Cochrane, é mandatório que se verifique pesquisa em pelo menos duas bases de pesquisa bibliográfica (idealmente, deverão ser pesquisadas duas bases generalistas e uma específica da área). No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, é altamente recomendado que uma das bases pesquisadas corresponda à CENTRAL ou a bases de ensaios clínicos como a ClinicalTrials.gov.*** ***Estudos de revisão da literatura em que a pesquisa decorra numa única base de dados não serão classificados como revisões sistemáticas.***	12 and 3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. **– MANDATÓRIO**	Appendix

		A query de pesquisa deve ser obrigatoriamente disponibilizada. A utilização de filtros de pesquisa da Inter-TASC é altamente recomendada (https://sites.google.com/a/york.ac.uk/issg-search-filters-resource/home)	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). **– MANDATÓRIO** ***As fases de selecção dos estudos primários devem ser descritas. Em consonância com as boas práticas da Cochrane, é mandatório que o processo de selecção envolva duas fases (fase de rastreio, em que os registos são seleccionados por título e abstract, e fase de inclusão, na qual se procede à leitura integral dos full texts). Em cada uma destas fases, o processo de selecção deve mandatoriamente envolver dois investigadores actuando de forma independente.***	12 and 3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. **– MANDATÓRIO** ***Trata-se de descrever de que forma se procedeu à extracção de dados dos estudos primários. Em consonância com as boas práticas da Cochrane, tal processo deverá envolver dois investigadores de forma independente.***	13 and 1
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. **– MANDATÓRIO** ***Trata-se de descrever as variáveis para as quais foi obtida informação.***	13 and 1
Risk of bias in individual studies / Risk of bias across studies	12/ 15	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. **– MANDATÓRIO** ***Em todas as revisões sistemáticas, deverá existir um processo de avaliação da qualidade dos estudos primários. No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, a aplicação dos critérios de risco de viés (Risk of Bias) da Cochrane é altamente recomendada. No caso de revisões sistemáticas de estudos observacionais, poderão ser seguidos os critérios ROBINS ou os critérios dos National Institutes of Health (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).***	13 and 2 14 and 1-2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). **– FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE**	*Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis. **– FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE**	*
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	*
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. **– MANDATÓRIO**	16 and 1

Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. **– MANDATÓRIO**	16 and 2
Risk of bias within and across studies	19/ 22	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). **– MANDATÓRIO**	16 and 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	*
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency. **– FACULTATIVO. MANDATÓRIO APENAS SE FOR FEITA META-ANÁLISE**	*
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	*
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). **– MANDATÓRIO**	17 and 1-2
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). **– MANDATÓRIO**	18 and 1
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research. **– MANDATÓRIO**	20 and 1
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. **– SEGUIR RECOMENDAÇÕES DA REVISTA**	20 and 2

Appendix

Search strategy: MEDLINE

1- NIPT[tiab] OR BambniTest[tiab] OR Harmony[tiab] OR informaSeq[tiab] OR MaterniT21 PLUS[tiab] OR NIFTY[tiab] OR
Panorama[tiab] OR PrenaTest[tiab] OR verify[tiab] OR VisibiliT[tiab] OR PRENATAL DIAGNOSIS[tiab] OR Non- invasive prenatal
test[Mesh] OR CELL-FREE DNA [Mesh])

2- AND (CHROMOSOM*[tiab] OR TRISOMY[tiab] OR ANEUPLOIDY[Mesh] OR DOWN[tiab] OR EDWARDS[tiab] OR PATAU[tiab])

3- AND (randomized controlled trial[pt] OR randomized controlled trials as topic[mh] OR random allocation [mh] OR double-blind
method[mh] OR single-blind method[mh] OR random*[tw] OR "Placebos"[Mesh] OR placebo[tiab] OR ((singl*[tw] OR doubl*[tw]
OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw] OR dumm*[tw])))

4- AND ("Sensitivity and Specificity"[MESH] OR predict* OR diagnose* OR diagnosi* OR diagnost* OR accura* OR sensitiv* OR
specific*)

5- 1 and 2 and 3 and 4