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Secondary non-invasive prenatal screening for fetal trisomy: an effectiveness study in a public health setting

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Objective To evaluate the effectiveness of secondary screening using non-invasive prenatal testing (NIPT) in a routine NHS setting including test performance, turn-around times (TATs) and no-call (failure to obtain result) rates. To examine the influence of maternal and fetal characteristics on test performance.

Design Retrospective cohort.

Setting London teaching hospital.

Sample A total of 8651 pregnancies undergoing screening for fetal trisomy using NIPT provided by an NHS cell-free DNA screening laboratory – the SAFE laboratory.

Methods Screening test evaluation and TATs. Univariate and multivariate logistic regression analysis to identify significant predictors of no-call results and reported by low fetal fraction (<2%), very high fetal fraction (>40%) and processing failure.

Main outcome measures Test performance, TATs and no-call rates, factors affecting no-call results.

Results Average TAT was 4.0 days (95% CI 4.0–4.2 days). Test sensitivities for trisomies 21 and 13/18 were 98.9% (95% CI 95.9–99.9%) and 90.4% (95% CI 80.0–96.8%), respectively. The overall no-call rate was 32/8651 (0.37%, 95% CI 0.26–0.52%). The overall risk of a no-call result was influenced by gestational age, dichorionic twin pregnancy, history of malignancy and pregnancies affected by trisomy 13/18, but not by maternal weight or use of low-molecular-weight heparin.

Conclusions High-throughput NIPT can be effectively embedded into a public health NHS setting. TATs of 4 days and no-calls of <0.5% were well within clinically desirable tolerances. Gestational age, maternal weight, assisted reproductive techniques, use of low-molecular-weight heparin and past history of malignancy did not have major impacts on test no-call rates and should not constitute reasons for withholding the option of NIPT from women.

Keywords Cell-free DNA, failed sample, fetal fraction, first-trimester screening, no-call rate, non-invasive prenatal testing, trisomy 13, trisomy 18, trisomy 21, twin pregnancy.

Tweetable abstract Turn-around times of 4 days, no-call (test failure) rates of 0.37% and highly accurate NIPT can be successfully embedded in the NHS.

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What we already know

- Commercial and research studies have demonstrated that NIPT is effective in both high-risk and low-risk populations.

SAFE test collaborative authors are in Appendix A.

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What this study adds

- High-throughput NIPT can be successfully embedded in a public health setting.
- Screening performance of NIPT in the NHS matches that published in research settings.
- Test turn-around times of 4 days and no-call rates of 0.37% are well within clinically desirable tolerances.
- Gestational age, maternal weight, assisted reproductive techniques, use of LMW heparin and history of malignancy have a negligible influence on test no-call rates.

Introduction

The United Kingdom National Screening Committee recommended a secondary screening model where women with a high-chance (≥1:150) combined or quadruple test result for fetal trisomy were to be offered non-invasive prenatal testing (NIPT), invasive testing or expectant management.[1] Despite a large number of studies reporting on NIPT, most are not comparable to a public health setting as they have been conducted in a research context[2–4] undertaken by private laboratories with incomplete reporting of maternal variables and pregnancy outcomes[5,6] or largely undertaken in low-risk populations.[7] Furthermore, there is a paucity of data on the impact of various maternal and pregnancy characteristics on routine NIPT performance in secondary screening. In view of these limitations, National Health Service England and Public Health England recommended in 2018 that an evaluative role of secondary NIPT screening, as outlined in the United Kingdom National Screening Committee recommendations, should be commissioned to establish specifically test turn-around times (TATs), rates and reasons for no-calls (failure to obtain a result) as well as test performance in the detection of the three common fetal trisomies.[1] We report on the performance of secondary NIPT screening conducted entirely in a UK public health setting from a large cohort of pregnancies. The aim of this study is first, to evaluate the effectiveness of NIPT in a routine National Health Service (NHS) setting with regards to test performance, TATs and no-call rates; and second, to examine the effects of maternal and fetal characteristics, obstetric history and medical history on no-call results.

Methods

St George's University Hospitals NHS Foundation Trust implemented a non-invasive prenatal test (the SAFE test) laboratory service in November 2015. The data for this study were derived from a retrospective analysis of prospectively collected data from the clinical implementation of the SAFE test in secondary screening for fetal trisomy between January 2016 and March 2019. As such, patients were not involved in the development of the research and no funding was necessary to undertake the analysis. Samples originated from secondary screening for a high-chance (>1:150) combined screening result or a screen-positive quadruple test in the SAFE test collaborative network (a total of 14 NHS trusts) and from women electing to undertake self-pay NIPT through NHS or private healthcare providers after either a low chance (<1:150) combined test result or no prior screening (a total of 13 providers). Referral centres reported the high-chance combined or quadruple test result, without details of specific biochemical or nuchal translucency measurements and only occasionally reported the presence of major fetal abnormalities. Pregnancies with very high risks (>1:10) were not excluded from SAFE test analysis as this was not an automatic indication for invasive testing. Healthcare professionals were encouraged to undertake the free online training module *Non-Invasive Prenatal Testing (NIPT): An Introduction for Healthcare Professionals* before sending samples to ensure adequate counselling and result interpretation.[8]

Blood was collected in a single 10-ml STRECK (Cell-free DNA BCT CE) tube labelled with three patient identifiers. This was stored and transported to the laboratory for processing at room temperature (6–37°C). The SAFE test procedural workflow is CE-IVD certified and was divided into the following steps: plasma separation and cell-free fetal DNA (cfDNA) extraction; automated library construction; automated emulsion polymerase chain reaction; Library DNA sequencing and automated data analysis. This resulted in a processing time of 35 hours for up to 32 samples, in multiples of eight, for optimal throughput.[9] The ensuing fragment count data were used as the input to a set of mixture models, which incorporated distributions of expected values for both trisomy-affected and unaffected samples. The models generated test likelihood ratios to quantify the relative likelihood that the DNA fragment count data supported a trisomy-affected or unaffected result for each trisomy under consideration.

Validity checks also took place for each sample, which ensured that the aligned fragment count was sufficient for the likelihood mixture model to be used, and second that the proportion of cfDNA in the sample that was of fetal origin was sufficient (>2%) for a result to be reported. For a fetal fraction between 2 and 4%, the SAFE test used a dynamic range test, which applied an algorithm using fetal fraction, depth of sequencing and a priori trisomy risk to

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determine the fetal fraction threshold to permit reporting.[10] A no-call result was also issued for a high cfDNA fetal fraction of >40% and a technical failure (failed chip, not enough plasma, low fragment count). In response to a no-call result, we undertook a repeat test by using the same blood sample – not by requesting a re-draw. The advantage of using the Ion Proton device is that only small volumes are required for sequencing, thereby permitting a rerun using spare stored serum that can be completed often within 3 days.

Outcome measures

Maternal and pregnancy characteristics, results of the cfDNA testing and pregnancy outcome were obtained from the SAFE test and outcome database. Details collected included maternal demographic characteristics, method of conception (spontaneous or artificial reproductive technology), maternal history (including previous malignancy), medication (including low-molecular-weight heparin [LMWH]) and sample origin (UK or non-UK based). Gestational age and chorionicity (in case of twin pregnancy) were determined by ultrasound performed by the referring centre. A vanishing twin was classified as ultrasound evidence of two gestational sacs with only one viable fetus identified. All referring centres used an internet portal for requesting tests and accessing results. The portal was also used to ascertain pregnancy outcome for the purposes of mandatory reporting on screening audits. The pregnancy outcomes were divided into non-trisomic, trisomy 21 (T21), T18 or T13 by either confirmed karyotype (n = 219) or phenotypical normality of the neonate. All abnormal karyotype results were cross checked with regional cytogenetic registers to ensure accuracy. Other core pregnancy outcomes, unrelated to NIPT, were not available as these were not routinely collected. Pregnancies lost to follow up or with incomplete reporting of outcomes were not included in the analysis.

Statistical analysis

Descriptive data were presented in median and interquartile range for continuous variables and in numbers and percentages for categorical variables. Comparisons between groups were performed using the Mann–Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. In the combined data of those with and without no-call results, univariate logistic regression analysis was used to determine which of the factors among maternal weight, height, history of malignancy, use of LMWH, gestational age at sampling, method of conception, origin of sample, type of pregnancy (singleton or dichorionic or monochorionic or vanishing twin) and fetal karyotype (non-trisomic or T21 or T18/13) were significant predictors of no-call test results (n = 32). To reduce the risk of over modelling the data, combinations of these factors were used in multivariate logistic regression analysis, where complete data were available (n = 3347), to determine the two/three most significant predictors of no-call results. For subgroup analysis, this process was repeated for no-call results secondary to low fetal fraction (n = 18). Due to the low number of cases of no-call results secondary to high fetal fraction (n = 9) and technical problems (n = 5), a univariate analysis using Fisher's exact and Mann–Whitney U test was performed. The statistical software package SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad (GraphPad Software, San Diego, CA, USA) were used for data analyses.

Results

Study population

Between January 2016 and March 2019, the SAFE Test laboratory analysed 8655 samples, of which 8651 had outcomes (Table 1). The performance characteristics of the SAFE test are shown in Table 2. The average TAT for the

Table 1. Maternal and pregnancy characteristics of the tested population

Characteristic	Tests with results (n = 8651)
Maternal height (cm)	165 (160–170)
Maternal weight (kg)	65.0 (58.6–75)
Maternal age (years)	34.6 (31.1–38.1)
Gestation at sampling (weeks)	12.0 (11.0–14.0)
Conception	
Spontaneous	7881 (91.1%)
Artificial reproductive technology	770 (8.9%)
Medical history	
History of malignancy	23 (0.3%)
Use of low-molecular-weight heparin	248 (2.9%)
Sample origin	
UK	7812 (90.3%)
Non-UK	837 (9.7%)
Type of pregnancy	
Singleton	8289 (95.8%)
Dichorionic twin	213 (2.5%)
Monochorionic twin	41 (0.5%)
Vanishing twin	108 (1.2%)
Number of trisomy pregnancies	
Trisomy 21	167 (1.9%)
Trisomy 13/18	52 (0.6%)

Data presented as median (interquartile range) or number (%).
T21, Down syndrome; T18/13: Edward and Patau syndromes.

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Table 2. Performance of the SAFE test

	Number (%), Number (%, 95% CI) or median (IQR)
Indication	
High-chance prior screen	3017 (35%)
Self-pay tests	5634 (65%)
Turn-around time (days)	4 (4–4.2)
Retesting rate	164, (1.9%, 1.6–2.2%)
No-call rate	
Overall	32, (0.37%, 0.26–0.52%)
Low fetal fraction	18, (0.21%, 0.13–0.33%)
High fetal fraction	9, (0.10%, 0.05–0.20%)
Technical failure	5, (0.06%, 0.02–0.14%)
Test performance	
Overall false positive rate	10, (0.12%, 0.02–0.22)
T21 sensitivity	173/175 (98.9%, 95.9–99.9)
T21 positive predictive value	96.7% (92.8–98.4%)
T18/13 sensitivity	47/52 (90.4%, 80.0–96.8%)
T18/13 positive predictive value	92.2% (81.5–96.9%)

Data presented as median (interquartile range) or number (%).
IQR, interquartile range; T21, trisomy 21, T18/13, trisomy 18 or 13.

SAFE test was 4.0 days (interquartile range 4.0–4.2 days) with only 2 days of unplanned downtime out of 1094 service days. The test sensitivities for T21 and T13/18 were 98.9% (95% CI 95.9–99.9%) and 90.4% (95% CI 80.0–96.8%), respectively. The equivalent positive predictive values for T21 and T13/18 were 96.7% (95% CI 92.8–98.4%) and 92.2% (95% CI 81.5–96.9%), respectively. The overall no-call rate following a second attempt of laboratory processing was 32/8651 (0.37%, 95% CI 0.26–0.52%). The majority of T21 or T13/18 pregnancies were in the high-chance population rather than the self-selected referrals. There were 110 (66%) T21 and 38 (73%) T13/18 from the high-chance group and 57 (33%) T21 and 14 (27%) T13/18 from the self-pay group. In pregnancies identified as high-chance (n = 252), 177 (70%) had prenatal invasive testing, with the remainder opting for post-natal confirmation by phenotypical normality of the neonate or karyotype if trisomy was suspected. The rates of pregnancies involving T21 and T13/18 in those with no-call results were 2/32 (6.3%, 95% CI 0.8–22.6%) and 4/32 (12.5%, 95% CI 3.4–32.0%), respectively. Of the women with no-call results, 7/32 (21.9%, 95% CI 10.7–39.0%) had prenatal invasive testing, 6/32 (18.8%, 95% CI 8.5–35.7%) had a successful blood re-draw and 19/32 (59.4%, 95% CI 42.2–74.5%) opted for post-natal confirmation of karyotype.

Factors influencing no-call results

Multivariate logistic regression analysis demonstrated that the overall risk of a no-call result was influenced by dichorionic twin pregnancy, history of malignancy and pregnancies affected by T18/13, but not maternal weight or use of LMWH (Table 3). Low fetal fraction resulted in a no-call result in 18/8651 samples (0.21%, 95% CI 0.13–0.33%). Multivariate logistic regression analysis demonstrated that the risk of a no-call result due to low fetal fraction was higher with increased maternal weight (Figure 1), in dichorionic twins and in pregnancies affected by T18 or T13 (see Supplementary material, Table S1). No-calls due to high fetal fraction occurred in 9/8651 (0.10%, 95% CI 0.05–0.20%) pregnancies and was influenced by maternal height, gestational age and maternal history of malignancy (see Supplementary material, Table S2). Technical reasons for no-calls occurred in 5/8651 (0.06%, 95% CI 0.02–0.14%) samples, which occurred more frequently in samples originating from outside the UK and in pregnancies affected by T18 or T13 (see Supplementary material, Table S2).

Discussion

Main findings

This study demonstrates the feasibility and effectiveness of a high-throughput NIPT secondary screening service embedded in a public health NHS setting. The screening performance of the SAFE test with sensitivities of 98.9% for T21 and 90.4% for T18/13, are comparable to those from studies published in research settings or in commercially funded trials. The SAFE test also performed well with a median TAT of 4 days, and only 2 days of unplanned downtime in over 3 years of service. In a secondary screening model, NIPT is offered as an alternative to expectant management and invasive prenatal testing after a primary screening by first-trimester combined test or early second-trimester maternal serum biochemical screening. The latter care pathway inevitably leads to NIPT being performed at a more advanced gestation compared with the use of NIPT as a primary screening test. In the clinical context of secondary screening, fast TATs and fewer laboratory downtime days are an essential attribute for a high-throughput NIPT laboratory.

Strengths and limitations of the study

This is one of the first studies to evaluate the effectiveness of secondary NIPT screening in a large population of women undergoing routine care in a non-research setting and therefore offers unique and pragmatic insights into practical issues regarding the implementation of cfDNA screening in a public health system. Although a large study, the very low overall test no-call rate made it difficult to validate previously reported modulators of the test no-call rate. For instance, maternal history of malignancy was linked to only one no-call, resulting in an odds ratio with large confidence interval and low level of precision. There is also the possibility that no-call variables may be different

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Table 3. Univariate and multivariate logistic regression analyses of factors from maternal and pregnancy characteristics in the prediction of overall no-call results (n = 32) in 8651 (univariate) and 3348 (multivariate) pregnancies

Variable	Univariate analysis Odds ratio (95% CI)	*P* value	Multivariate analysis Adjusted Odds ratio (95% CI)	*P* value
Maternal height (cm)	0.987 (0.939–1.038)	0.611	x	x
Maternal weight (kg)	1.018 (0.999–1.037)	0.059	x	x
Gestational age	1.091 (1.021–1.165)	**0.010***	x	x
Medical history				
ART pregnancies	1.657 (0.579–4.741)	0.346	x	x
History of malignancy	13.754 (1.723–109.800)	**0.013***	17.265 (2.144–139.036)	**0.007***
Use of LMWH	1.600 (0.217–11.796)	0.645	x	x
Sample origin				
Non-UK	2.162 (0.887–5.268)	0.090	x	x
Type of pregnancy				
DC twin	4.142 (1.252–13.704)	**0.020***	5.275 (1.565–17.785)	**0.007***
MC twin	–	–	x	x
Vanishing twin	–	–	x	x
Fetal karyotype				
T21	3.414 (0.809–14.405)	0.095	x	x
T13/T18	24.974 (8.442–73.880)	**<0.001***	33.029 (10.616–102.762)	**<0.001***

The following were used as reference populations: spontaneous conceptions for ART pregnancies, UK samples for non-UK samples, singleton for twin pregnancies and non-trisomic fetuses for trisomic fetuses.
(Bold)* indicates statistically significant result (p<0.05).
–, OR not calculated due to lack of no-call cases; ART, artificial reproductive technology; DC, dichorionic; LMWH, low-molecular-weight heparin; MC, monochorionic; T21, Down syndrome; T18/13: Edward and Patau syndromes.

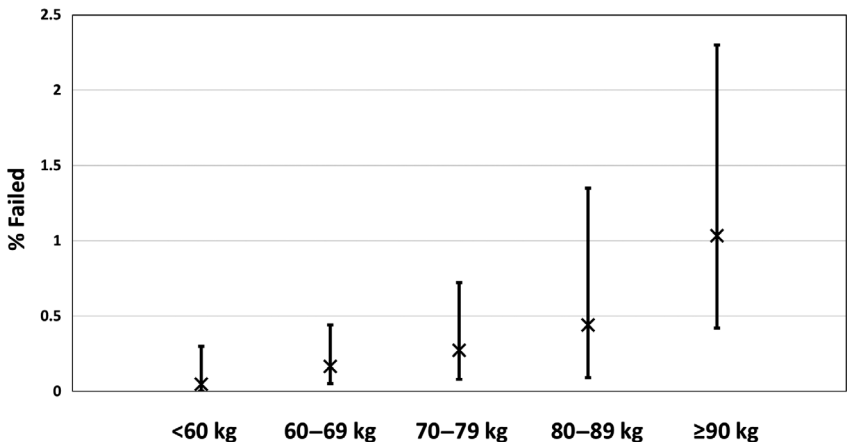

Figure 1. Cross and whisker plots showing the no-call rate for low fetal fraction with maternal weight in 10-kg blocks for the cohort of 8651 tests.

in a low-risk population, such as self-referring women who may already have a low chance screening result. Although further data will be needed to confirm the significance of patient characteristics related to no-calls, it is reassuring to know that they resulted in very few test failures. Although we are able to report on the number of women referred to our centre for invasive testing in the studies population, we do not have access to data on women who were not referred for invasive testing or who were referred elsewhere for further management. Therefore, we are unable to report the effect of secondary screening on the overall screening programme nor the potential weakness of a secondary versus primary NIPT screening in reducing the false-positive rate at the cost of a slight reduction in the population detection rate.

Interpretation

Low fetal fraction no-calls

The majority of institutional and societal guidelines recommend that fetal fraction is measured when undertaking NIPT to ensure that fetal DNA is present in sufficient

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quantity in the maternal sample to enable a technically reliable estimate of fetal trisomy risk.[11,12] Most NIPT providers set a fixed fetal fraction threshold (usually 4%) below which a no-call result is issued. The consequence of a set reporting threshold is that these NIPT tests usually have a 2–5% no-call rate. The no-call rate for low fetal fraction with the SAFE test (0.21%) is much lower than previously reported for NIPT that measure fetal fraction. The latter is almost certainly due to the use of a dynamic range that allows reporting down to fetal fractions as low as 2.5% – as long as the fragment count is high enough to provide reliable likelihood ratios for fetal trisomy. The lower no-call rate is an important attribute in a secondary screening programme of high-chance women to minimise any delays in providing NIPT results in women who are further advanced in pregnancy compared with primary NIPT screening. It is also reassuring to find that the relative lower no-call rate is not associated with a concomitant drop in the sensitivity and specificity for fetal trisomy.

Low fetal fraction no-calls were more likely to occur with increased maternal weight, dichorionic pregnancy and trisomic pregnancies – consistent with the findings of previous review publications.[13,14] Although the odds ratios for these factors varied from eight-fold up to 36-fold, the absolute probabilities of no-call results were low. For example, the risk of a no-call result was only 1% in women weighing over 90 kg (Figure 1). Similarly, the overall risk of a no-call in a dichorionic pregnancy was 1.4%. Importantly, low fetal fraction no-call results were more likely in pregnancies affected by T18 and T13, in keeping with previous evidence[15] and probably as a consequence of the smaller placental size and lower levels of circulating fetal cfDNA in these pregnancies. Due to the increased risk of no-call results in trisomic pregnancies, consideration should be given to offer invasive testing to these women. Reassuringly, use of LMWH did not result in higher no-call rates – contrary to a previous report that the majority of no-calls were from women on LMWH.[16] The authors suggested that plasma samples had curiously elevated levels of short fragment length cfDNA that biased test results. An alternative hypothesis for the putative test failure is that LMWH is a known inhibitor of polymerase chain reactions, which may reduce the amount of DNA amplification required in the NIPT process.[17] However, our study suggests that women may use LMWH without affecting cfDNA test no-call rates or performance. The caveat to this conclusion is that the majority of the women in this study were on lower dose prophylactic LMWH rather than high-dose therapeutic LMWH – for which there is possibly a higher risk of a no-call result.[18]

Technical and high fetal fraction no-calls

Only samples from non-UK based centres had an increased risk of technical failure resulting in no-calls. This is a centre-specific finding that may be due to the technique of sampling, handling of samples, massive temperature fluctuations and delays in transport. The risk of a technical failure was also higher with T18/13, but this finding is based on only one no-call test result. Similarly, a solitary case contributed to an increase in high fetal fraction no-call associated with a maternal history of malignancy. The concentration of circulating cfDNA is known to be increased in patients with malignant disorders, probably as a consequence of increased cell turnover and death and release of cfDNA into the circulation.[19]

Conclusions

High-throughput NIPT can be effectively undertaken and clinically embedded into routine NHS practice. Screening performance of NIPT in the NHS setting matches that published in research settings. Test TAT of 4 days and low no-call rate of <0.5% were well within expected clinical tolerances. Gestation, maternal weight, assisted reproductive techniques, LMWH use and history of malignancy did not have major impacts on test no-call rates and should not constitute reasons for withholding the option of NIPT.

Disclosure of interests

Yourgene is contracted to supply the IONA system to St Georges University Hospitals NHS Foundation Trust as the basis of the SAFE test. None of the authors have pecuniary interests in Yourgene or the SAFE test service. The authors have no other disclosures. Completed disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

Conceptualisation: BT, GPG; Methodology: BT, GPG; Data collection: GPG, JH, RD, KP, JS; Statistical analysis: GPG; Data interpretation: GPG, BT; Manuscript draft: GPG, BT; Manuscript review and editing: GPG, JH, RD, KP, JS, BT.

Details of ethics approval

This retrospective study of routinely collected clinical data was collated from ongoing continuous laboratory audit and was deemed not to require ethics approval or signed patient consent. Patients and the public were not involved in the design of this study.

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None.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table S1. Univariate and multivariate logistic regression analyses of factors from maternal and pregnancy characteristics in the prediction of no-call results due to low fetal fraction ($n = 18$). The following were used as reference populations: spontaneous conceptions for assisted reproductive technique pregnancies, UK samples for non-UK samples, singleton for twin pregnancies and non-trisomic fetuses for trisomic fetuses.

Table S2. Univariate analyses of factors from maternal and pregnancy characteristics in the prediction of no-call results due to high fetal fraction and technical failure. The following were used as reference populations: spontaneous conceptions for assisted reproductive technique pregnancies, UK samples for non-UK samples, singleton for twin pregnancies and non-trisomic fetuses for trisomic fetuses. ■

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Appendix A

Collaborators

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