High-Risk Case Results from a Quality Assurance (QA) Program Monitoring Single-Nucleotide Polymorphism (SNP)-Based NIPT Performance

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Introduction

- Non-invasive prenatal testing (NIPT) was introduced as a screening tool for chromosomal conditions in 2013, and its uptake in the clinical setting has been rapid.
- Numerous analytical and clinical validation studies have demonstrated high performance of the single-nucleotide polymorphism (SNP)-based NIPT methodology.[1,2,3]
- Few publications detail the features and results of laboratory quality assurance (QA) programs.
- This study aims to demonstrate that a laboratory can successfully monitor test performance through a self-driven, QA program.
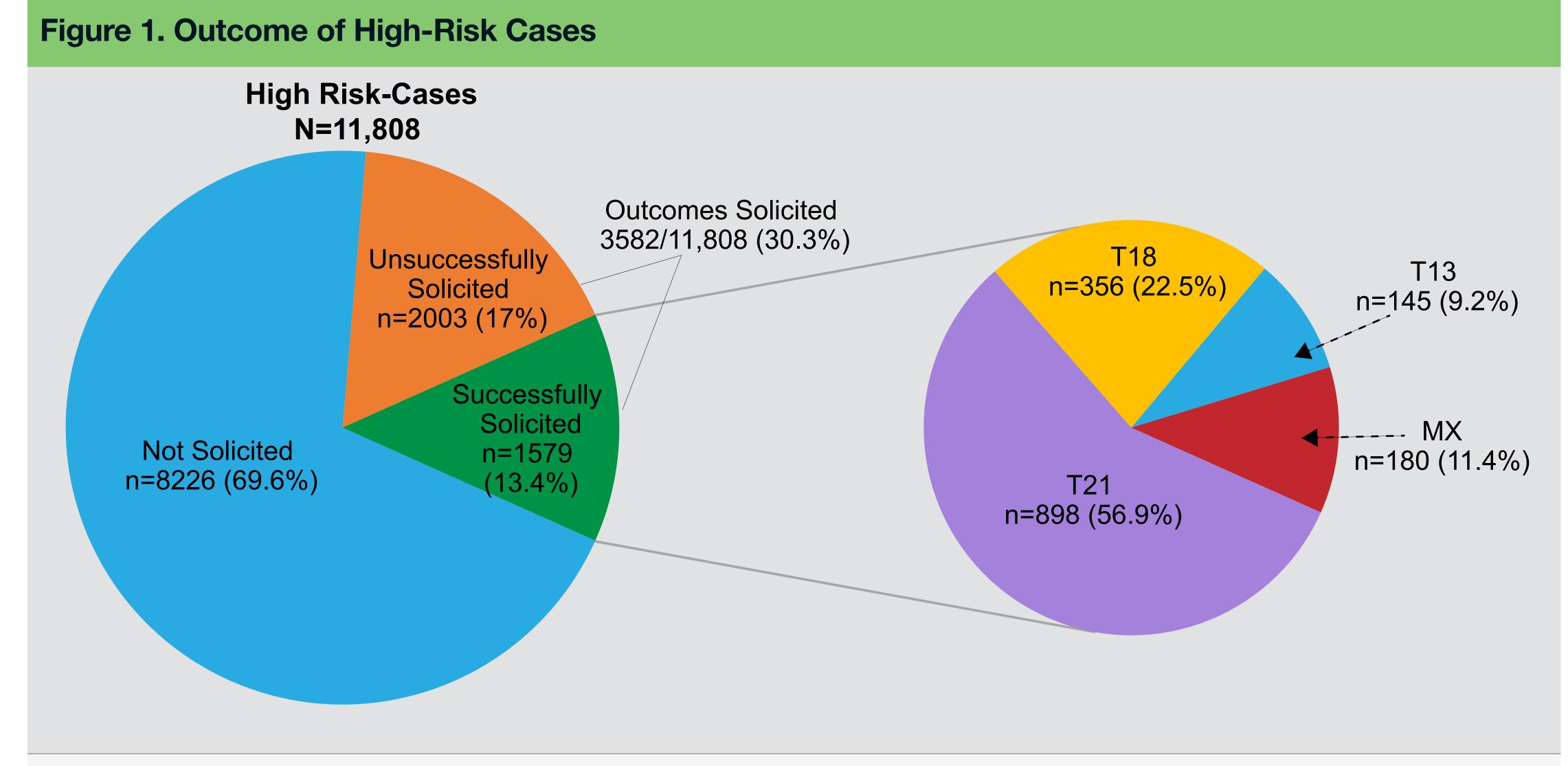
Objective

- To review pregnancy outcome data collected as part of a prospective QA laboratory-based program and assess the performance of a SNP-based NIPT for detection of aneuploidies.

Study Design

- Among 926,751 SNP-based NIPT tests performed between October 1, 2013 to June 30, 2017 at a single reference laboratory, fetal outcomes were solicited for cases that received a high-risk result (defined as aneuploidy risk ≥1%) for trisomy 21, 18, 13 (T21, T18, T18), and/or monosomy X (MX).
- Outcome information was collected by genetic counselors using a standard procedure of up to 3 requests by telephone, email, and/or facsimile, and verified by medical records when available.
- NIPT results were considered true positive if confirmed by pre/postnatal karyotype or if prenatal ultrasound findings indicated either one major criteria or two minor criteria.
- Positive predictive values (PPV) were calculated for samples with clinical truth obtained through clinic solicitation; calculated PPV values were compared with previously published test performance.[1]
- Fisher's exact test was performed to compare the PPVs for T21, T18, T13 and MX between women of advanced maternal age (≥35 years) and average maternal age (<35 years).
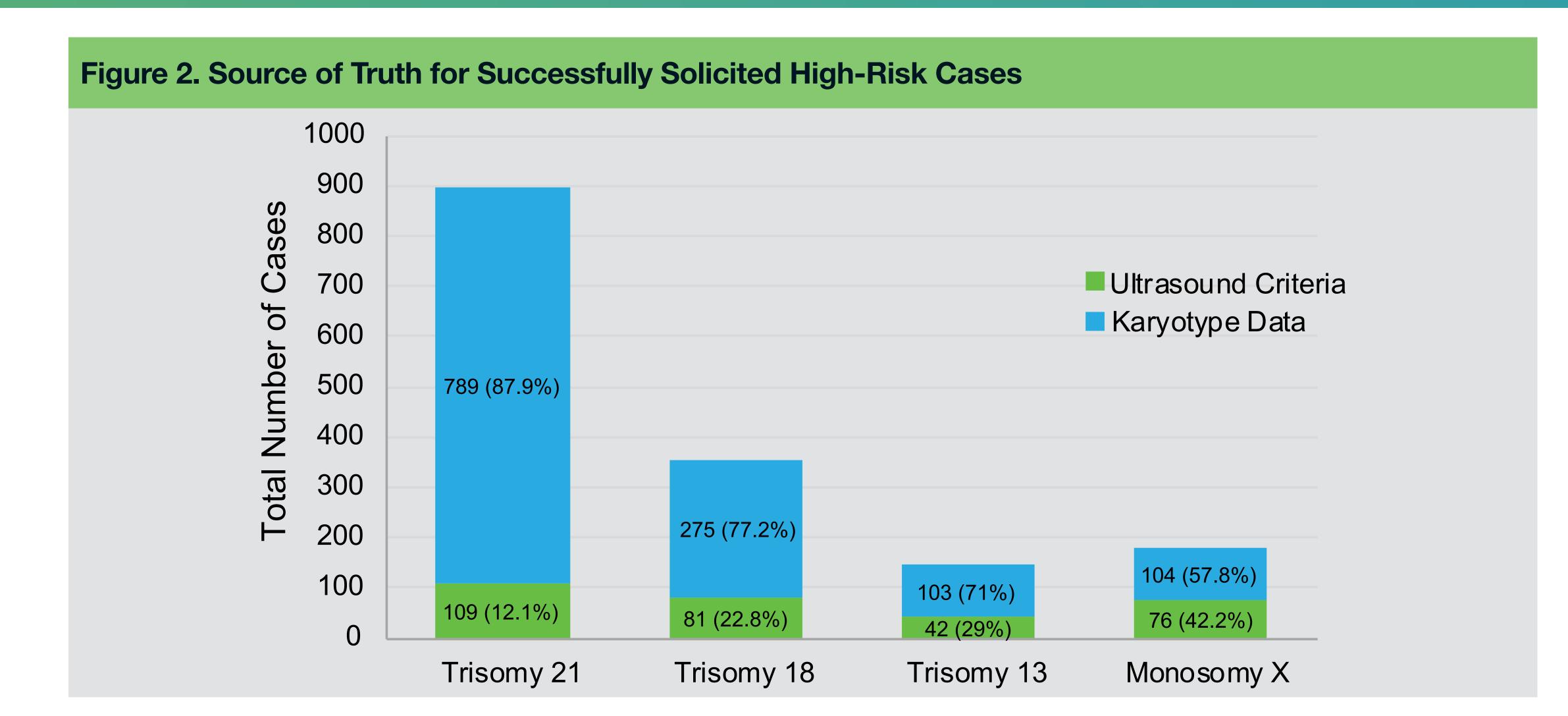
Results

- During the study period, results were reported for 926,751 cases at a single reference lab.
- A total of 11,808 (1.3%) cases received a high-risk result; of those, fetal outcome was solicited for 3,582 (30.3%) cases (Figure 1).
- Fetal outcome information was successfully obtained for 1,579/3,582 (44.1%) cases, who were at risk for T21(59.6%), T18 (22.5%), T13 (9.2%), and/or MX (11.4%) (Figure 1).

Figure 1. Outcome of High-Risk Cases
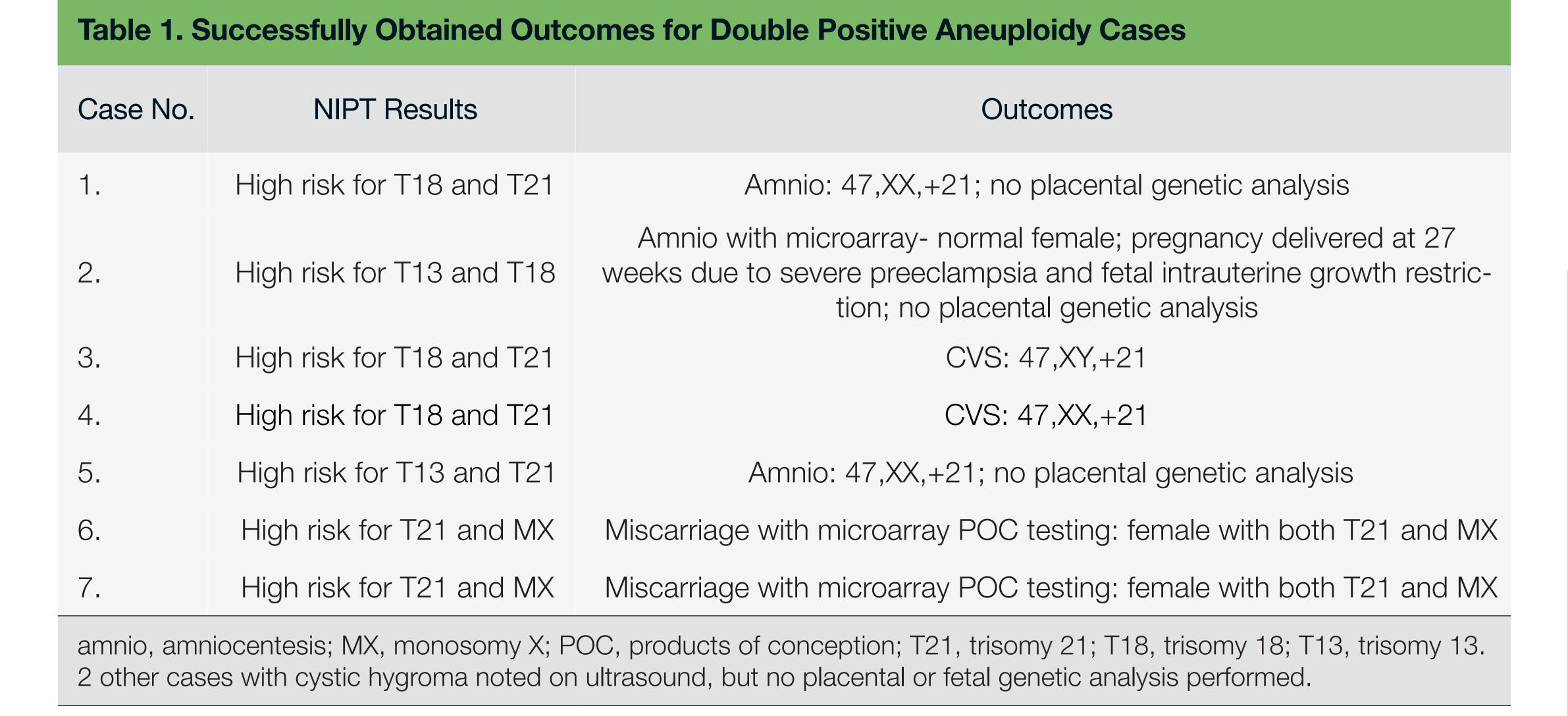

High Risk-Cases
N=11,808

T21, trisomy 21; T18, trisomy 18; T13, trisomy 13; MX, monosomy X.

- The average maternal and gestational ages for cases with outcome data were 35.3 years and 13.9 weeks, respectively, and the average fetal fraction for this cohort was 11.4%.
- Clinical truth was obtained based on either ultrasound criterial or pre/postnatal karyotype data (Figure 2).

Figure 2. Source of Truth for Successfully Solicited High-Risk Cases

- Of the 11,808 high-risk cases, 20 (0.17%) received a double positive aneuploidy result; of those, an attempt was made to obtain fetal outcome data for 9 cases and was successfully obtained for 7 cases (Table 1).

Table 1. Successfully Obtained Outcomes for Double Positive Aneuploidy Cases

Case No.	NIPT Results	Outcomes
1.	High risk for T18 and T21	Amnio: 47,XX,+21; no placental genetic analysis
2.	High risk for T13 and T18	Amnio with microarray- normal female; pregnancy delivered at 27 weeks due to severe preeclampsia and fetal intrauterine growth restriction; no placental genetic analysis
3.	High risk for T18 and T21	CVS: 47,XY,+21
4.	High risk for T18 and T21	CVS: 47,XX,+21
5.	High risk for T13 and T21	Amnio: 47,XX,+21; no placental genetic analysis
6.	High risk for T21 and MX	Miscarriage with microarray POC testing: female with both T21 and MX
7.	High risk for T21 and MX	Miscarriage with microarray POC testing: female with both T21 and MX

amnio, amniocentesis; MX, monosomy X; POC, products of conception; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13.
2 other cases with cystic hygroma noted on ultrasound, but no placental or fetal genetic analysis performed.

- Overall PPVs reported in this cohort were 95.2% (T21), 93.5% (T18), 77.9% (T13), and 86.7% (MX), and are consistent with or higher than a previous published report[1] (Table 2).

Table 2. Clinical Experience and Test Specifications of High-Risk Cases from Current and Prior Study

	Trisomy 21	Trisomy 18	Trisomy 13	Monosomy X	Total
High-Risk Cases-Current Study (October 1, 2013-June 30, 2017)					
Total number of high-risk cases reported, n (%)	7,040 (59.5)	1,921 (16.2)	1,098 (9.3)	1,769 (15.0)	11,828[a]
Number of cases with solicited follow-up, n (%)	2,103 (58.6)	711 (19.8)	302 (8.4)	475 (13.2)	3,591[b]
Number of cases with follow-up obtained, n (%)	898 (56.9)	356 (22.5)	145 (9.2)	180 (11.4)	1,579
PPV=TP/(TP+FP)	**95.2%** (855/898)	**93.5%** (333/356)	**77.9%** (113/145)	**86.7%** (156/180)	-
High-Risk Cases-Prior Study[1] (March 1, 2013-September 30, 2013)					
Number of cases with solicited follow-up, n (%)	233 (65.4)	55 (15.4)	30 (8.4)	38 (10.7)	356
Number of cases with follow-up obtained, n (%)	154 (69.3)	29 (13.1)	21 (9.5)	18 (8.1)	222
PPV=TP/(TP+FP)	**90.9%** (140/154)	**93.1%** (27/29)	**38.1%** (8/21)	**50.0%** (9/18)	-

[a]20 patients had double positive aneuploidy results; [b]9 patients had double positive aneuploidy results.
PPV, positive predictive value; TP, true positive; FP, false positive; FN, false negative.

- In our cohort, the PPV for T21 was significantly higher for women of advanced maternal age (≥35 years) compared with women of average maternal age (<35 years) (96.5% vs 92.4%; Fisher's test P=0.0113). The PPVs were not significantly different (p>0.05) between the ≥35 years and <35 years age groups for T18 (94.6% vs. 91.3%), T13 (81.6% vs. 73.9%) and MX (79.6% vs. 89.3%) (Table 3).

Table 3. Test Specifications of High-Risk Cases from Women of Average and Advanced Maternal Ages

	Trisomy 21	Trisomy 18	Trisomy 13	Monosomy X
High-Risk Cases-Current Study				
High-Risk with Clinical Truth, all maternal ages, n	898	356	145	180
High-Risk Cases-Current Study, Maternal Age <35				
High-Risk with Clinical Truth, n (%)	291 (32.4%)	115 (32.3%)	69 (47.6%)	131 (72.8%)
PPV=TP/(TP+FP)	**92.4%** (269/291)	**91.3%** (105/115)	**73.9%** (51/69)	**89.3%** (117/131)
High-Risk Cases-Current Study, Maternal Age ≥35				
High-Risk with Clinical Truth, n (%)	607 (67.6%)	241 (67.7%)	76 (52.4%)	49 (27.2%)
PPV=TP/(TP+FP)	**96.5%** (586/607)	**94.6%** (228/241)	**81.6%** (62/76)	**79.6%** (39/49)

PPV, positive predictive value; TP, true positive; FP, false positive; FN, false negative.

Conclusions

- This study demonstrates that a laboratory-driven QA program can monitor the performance of non-invasive prenatal screening for aneuploidy detection with acceptable response rates (44.1% in this cohort).
- The PPV of NIPT has been the subject of much speculation, however, these data suggest that PPVs observed in this study are congruent with previously published PPVs[1] and may even be higher than previously reported for T21, T18, T13, and MX.
- PPVs for T18, T13, and MX were not significantly different between women of advanced maternal age (≥35 years) and women of average maternal age (<35 years) in our cohort; although statistically significant, the difference in PPV for T21 in women between the two groups (≥35 years, 96.5% versus <35 years, 92.4%) is unlikely to be of clinical significance, supporting the use of NIPT as a primary screen for T21 and other common aneuploidies for women of all ages.
- Prospective monitoring of the performance of a laboratory test, or "QA", is the responsibility of a clinical molecular laboratory; the 44.1% follow-up rate for high-risk calls reflects dedication of both laboratory genetic counselors and the obstetrical care providers the laboratory serves.
- Our data detail critical information about SNP-based NIPT performance in a general unselected population that, in turn, can enable providers to counsel patients more accurately.

References

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