

BACKGROUND

Noninvasive prenatal testing (NIPT) for screening of common aneuploidies has become the standard of care in the past decade. Professional organizations such as American College of Medical Genetics and Genomics (ACMG), National Society of Genetic Counselors (NSGC), and Society of Maternal Fetal Medicine (SMFM) recommend informing all pregnant patients of the option of NIPT irrespective of a priori risks. Noninvasive prenatal testing (NIPT) for screening of common aneuploidies has become a standard of care in the United States since 2011 and is slowly being adopted globally. NIPT has demonstrated a high detection rate with a low false positive rate in screening for aneuploidies; a significant advantage over conventional serum screening methods. We have validated the Vanadis® NIPT test as a laboratory developed test (LDT) in our US lab and Malaysia lab. The Vanadis® NIPT is a non-PCR based, cost-effective, highly precise assay with a short turnaround time (7 days) for the effective screening of the common trisomies 13, 18 and 21. The high precision of the Vanadis® NIPT method results in a high detection rate also in low fetal fraction samples, reducing the no call rate compared to alternative methods. The Vanadis® NIPT system is based on fully automated, high yield counting of chromosome-specific DNA balls created via rolling circle DNA replication¹ (Figure 1).

METHODS

A total of 234 and 168 samples were utilized for a laboratory developed test (LDT) in the US lab (3 runs) and Malaysia lab (2 runs), respectively. These runs included SeraSeq T21 (n=14), T18 (n=6) and T13 (n= 6) reference materials along with sets of plasma samples. Plasma sample types included in this validation study are maternal plasma from confirmed trisomies, and from pregnancies negative for an aneuploidy. Assay technology is described in Figure 1. The following performance metrics were performed in this study: clinical sensitivity, clinical specificity, no-call rates, accuracy and precision. Quality assessment and automated data analysis was performed and samples were classified as either low or high risk based on Z score cutoffs of 3.5 for chromosome 21 and 3.15 for chromosomes 18 and 13 (Table 1). Samples failing quality assessment were classified as no-call. In addition to aneuploidy screening, 234 samples had fetal sex classification determined.

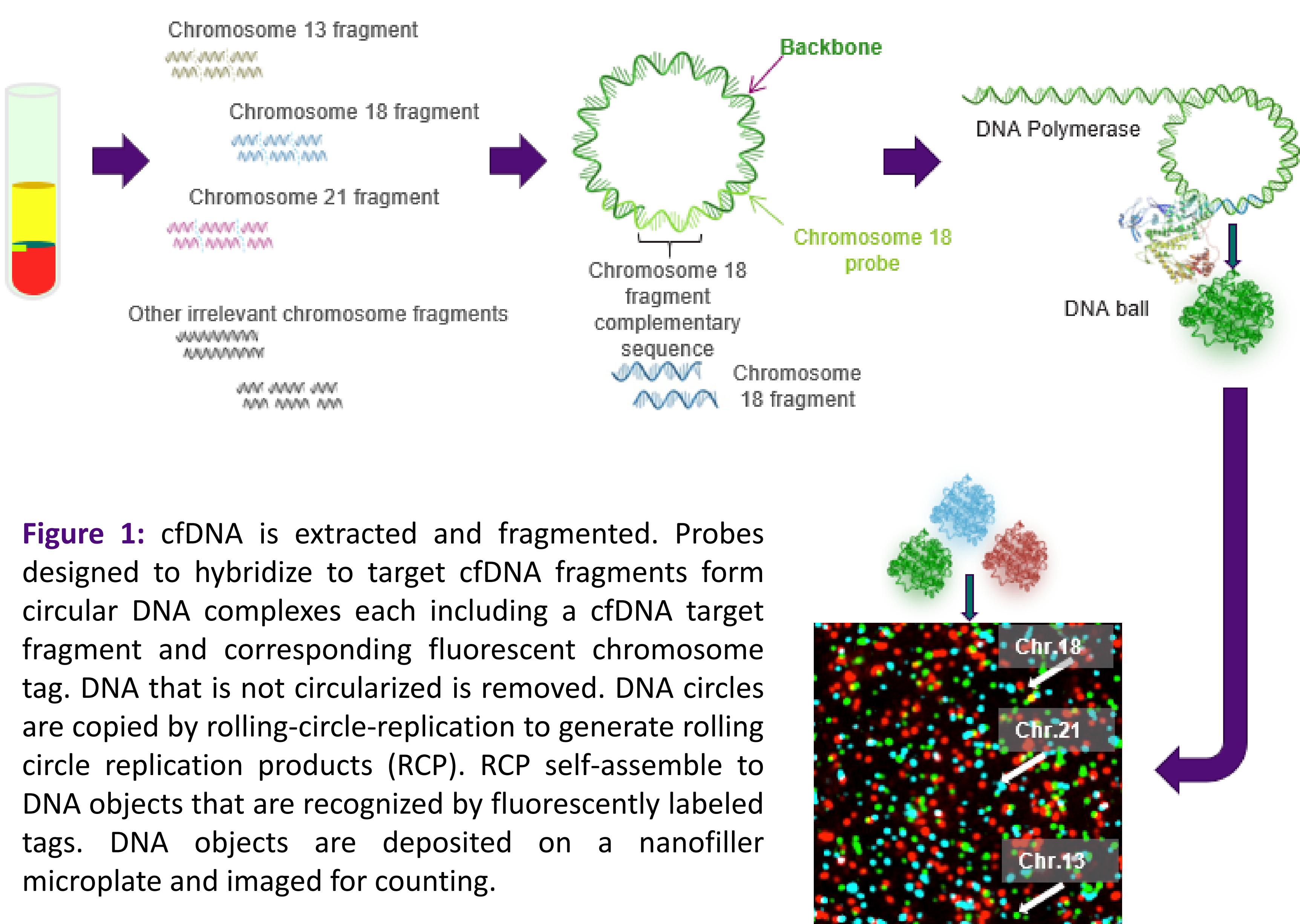


Figure 1: cfDNA is extracted and fragmented. Probes designed to hybridize to target cfDNA fragments form circular DNA complexes each including a cfDNA target fragment and corresponding fluorescent chromosome tag. DNA that is not circularized is removed. DNA circles are copied by rolling-circle-replication to generate rolling circle replication products (RCP). RCP self-assemble to DNA objects that are recognized by fluorescently labeled tags. DNA objects are deposited on a nanofiller microplate and imaged for counting.

REFERENCES

- Imaging Single DNA Molecules for high precision NIPT. Fredrik Dahl et al., Nature Scientific Reports, (2018), 8:4549

RESULTS

- Combined PKIG USA and Malaysia datasets:
 - Analytical specificity was determined to be 99.8% and analytical sensitivity was determined to be 100% for all 3 trisomies.
 - The overall combined Precision (Positive Predictive Value) for all 3 trisomies was assessed to be 92.9% (due to small number of true positives).
 - Negative predictive value (NPV) was 100% for the 3 trisomies.
 - SeraSeq Reference materials (T21, T18, T13) accuracy and precision as 100% (Table 2)
- Two samples had a “no-call” result for aneuploidy. Both no-call samples were normal controls.
- Fetal sex was accurately classified in 227/234 samples (Table 3)

Table 1. Analytical Specificity and Sensitivity

Condition	Number of Samples from PKIG USA and Malaysia Tested for T13, T18, T21				Performance Metrics					
	True Negative	False Positive	True Positive	False Negative	Total	Accuracy	Precision	Specificity	Sensitivity	Negative Predictive Value
Trisomy 13	433	1	6	0	440	0.998	0.857	0.998	1.000	1.000
Trisomy 18	434	1	6	0	441	0.998	0.857	0.998	1.000	1.000
Trisomy 21	428	0	14	0	442	1.000	1.000	1.000	1.000	1.000
Total	1295	2	26	0	1323	0.998	0.929	0.998	1.000	1.000

Table 2. Performance of True Positives

Sample	Ratio Chr21	Ratio Chr18	Ratio Chr13	Z-score Chr21	Z-score Chr18	Z-score Chr13
SeraSeq T13	0.98	0.98	1.04	-5.43	-6.47	10.24
SeraSeq T13	0.98	0.97	1.05	-5.36	-6.87	11.59
SeraSeq T18	0.99	1.02	0.99	-4.07	6.43	-2.26
SeraSeq T18	0.99	1.03	0.98	-3.62	8.22	-4.61
USA T21 Positive	1.06	0.97	0.97	17.57	-6.88	-6.78
USA T21 Positive	1.06	0.97	0.97	19.04	-8.42	-6.29
SeraSeq T21	1.04	0.97	0.99	12.24	-7.18	-2.64
SeraSeq T21	1.04	0.98	0.98	13.86	-6.43	-4.28
Malaysia T21 positive	1.07	0.96	0.97	24.26	-0.93	-7.45
Malaysia T21 positive	1.05	0.98	0.97	18.29	-6.88	-7.03
SeraSeq T21	1.04	0.98	0.99	13.49	-7.36	-3.42
SeraSeq T21	1.05	0.97	0.98	16.47	-8.98	-4.04

Z score threshold values: T13 ≥ 3.15; T18 ≥ 3.15; T21 ≥ 3.5

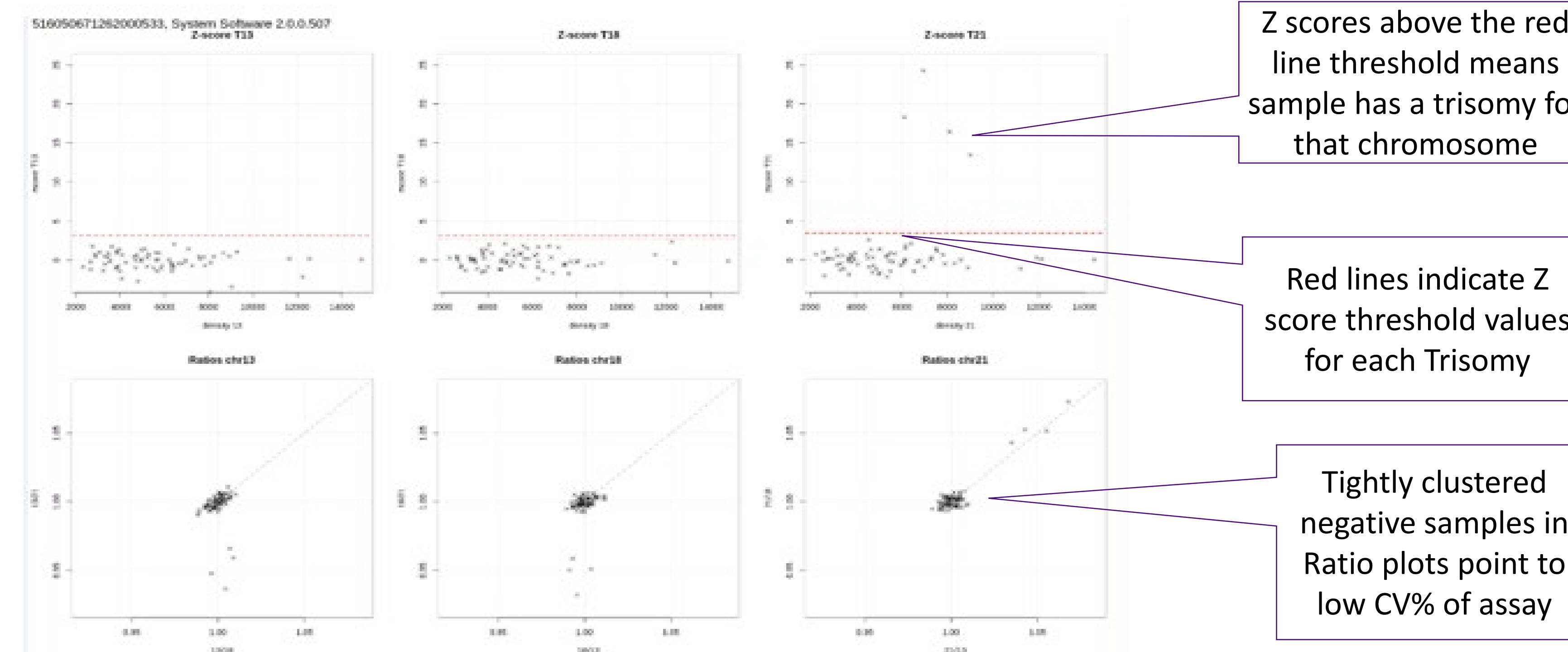


Table 3. Performance of Sex Determination

PERFORMANCE CRITERIA	Observed Results*	
	For Males	For Females
Accuracy	0.97	0.99
Precision	0.98	0.96
Analytical Specificity	0.97	0.97
Analytical Sensitivity	0.97	0.97

*7 out of 234 samples had border line ratio values and were miscalled for gender

DISCUSSION

- The Vanadis® assay is a novel rolling circle replication based method for NIPT testing and meets, and in some cases exceeds, the performance of PCR-based NIPT assays.
- High precision of this technology is established by eliminating PCR and high molecular counting of this assay does not rely on fetal fraction cutoffs (such as > 4%) thereby providing a minimal no-call rate.
- By complete automation and seamless bioinformatic reporting tools, this Vanadis® NIPT assay is well-suited to meet the needs for general population NIPT on a global scale.