CORRESPONDENCE

Rapid Nanopore Sequencing–Based Screen for Aneuploidy in Reproductive Care

TO THE EDITOR: Aneuploid pregnancies are a major cause of pregnancy loss, fetal structural anomalies, and developmental delays. Consequently, the identification of genetic abnormalities is an important component of prenatal and fertility care.[1] Existing testing methods include two main approaches: a rapid and targeted approach and a comprehensive and whole-genome approach. Rapid and targeted techniques, which include fluorescence in situ hybridization, multiplex ligation-dependent probe amplification, and quantitative polymerase-chain-reaction assays, test only a limited subset of chromosomes.[2] Comprehensive techniques include G-banded karyotyping, chromosomal microarray analysis, and next-generation sequencing.[2] Although the comprehensive techniques assess the entire genome, they typically take days to weeks to complete, require sending samples to high-complexity centralized laboratories for testing, and are costly to set up and perform, thereby limiting access to testing.

To address these limitations, we developed and validated a new short-read–based approach for library preparation, sequencing, and data analysis that enables accurate, inexpensive, and same-day genomewide aneuploidy detection with the use of a palm-sized, nanopore-based DNA sequencer[3-5] (Fig. 1, and Figs. S1 through S5 and Tables S1, S2, and S3 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Sequencing times and costs range from 10 minutes and $200 per sample for testing a single sample to 2 hours and less than $50 per sample when 10 samples are multiplexed and sequenced simultaneously. We termed this method short-read transpore rapid karyotyping (STORK).

Using STORK, we performed blinded testing of 218 sequential, remnant, reproductive specimens comprising products of conception after spontaneous pregnancy loss (64 specimens), chorionic villi after chorionic villus sampling (52 specimens), amniotic fluid from amniocentesis (50 specimens), and trophectoderm biopsy specimens from embryos undergoing preimplantation genetic testing for aneuploidy (PGT-A; 52 specimens). We compared the results obtained with STORK with those obtained with the use of standard clinical testing, which in this study represented the reference standard (Table 1 and Figs. S6, S7, and S8 and Tables S4 through S8). For products of conception, chorionic villi, and amniotic-fluid samples, STORK results represented the pregnancy karyotype with 100% accuracy (95% confidence intervals [CIs], 94.3 to 100, 93.2 to 100, and 92.9 to 100, respectively), and for PGT-A samples, STORK results were 98.1% concordant (95% CI, 89.7 to 100) with the clinical diagnosis of the embryos (Table 1). For 10 specimens of products of conception, results were discordant, but subsequent testing validated the results from STORK and the clinical di-

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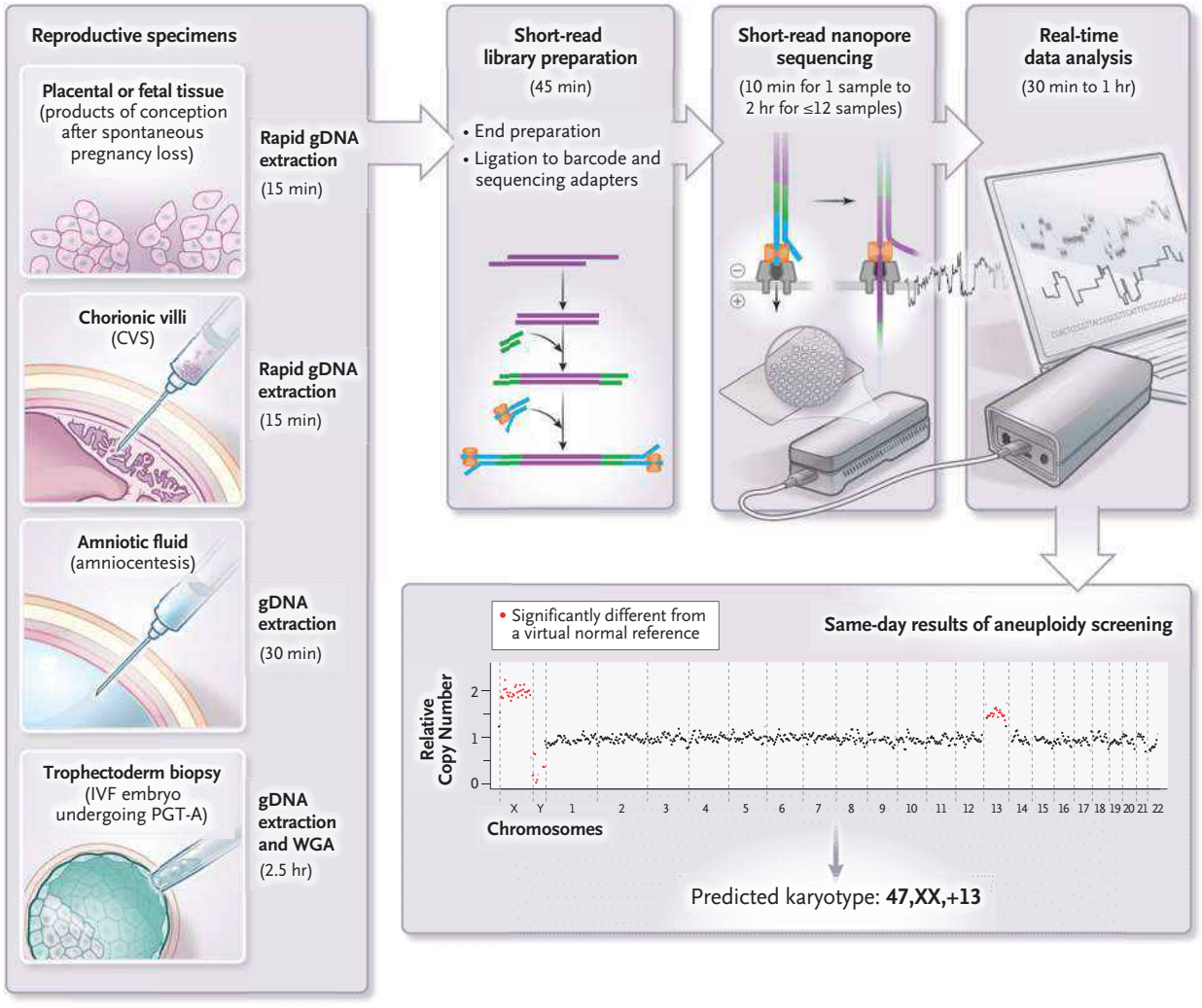

Figure 1. Workflow of Nanopore Aneuploidy Testing with STORK.

Shown is a universal method for rapid aneuploidy screening of reproductive specimens with the use of short-read transpore rapid karyotyping (STORK). Tissues and cells from chorionic villus sampling (CVS), amniocentesis, and products of conception from spontaneous pregnancy loss (miscarriage) were subjected to genomic DNA (gDNA) extraction. Trophectoderm biopsy specimens from embryos in an in vitro fertilization (IVF) procedure also underwent a standard whole-genome amplification (WGA) step. The gDNA was subjected to a nanopore short-read library preparation step for approximately 45 minutes and short-read nanopore sequencing for 10 minutes (1 sample) to 2 hours (≤12 samples). Sequencing data were collected and analyzed in real time, and results of aneuploidy testing on 24 chromosomes were returned within the same day. In the dot plot, each dot represents the relative copy number as compared with a virtual normal male reference (black dots indicate normal, and red dots indicate significantly different). A case with a STORK-predicted karyotype of 47,XX,+13 is shown. PGT-A denotes preimplantation genetic testing for aneuploidy.

agnosis was changed. To further validate the reproducibility of STORK, the laboratory technicians of a Clinical Laboratory Improvement Amendments–certified laboratory were trained in the use of the STORK assay. The technicians then independently performed the STORK assay on a separate set of 60 sequentially collected samples (20 specimens of products of conception, 20 specimens of chorionic villi from chorionic villus sampling, and 20 specimens of amniotic fluid from amniocentesis), and the results were compared with those obtained with the use of standard clinical testing. STORK results were 100% concordant (95% CI, 80.5 to 100) with those of standard clinical testing (Table S9). A full discussion of the results is provided in the Supplementary Appendix.

This study of nanopore-based sequencing of

Table 1. Performance Characteristics of the STORK Method on Reproductive Specimens.*

Sample Type	Sample Size	Results with STORK	Results with Standard Testing	Cases of Abnormality†	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	FDR	FNR
		no.	*no.*		*percent*						
Chorionic villi	52	52	52	10	100 (93.2–100)	100 (69.2–100)	100 (91.6–100)	100 (69.2–100)	100 (91.6–100)	0	0
Amniotic fluid	50	50	50	6	100 (92.9–100)	100 (54.1–100)	100 (92.0–100)	100 (54.1–100)	100 (92.0–100)	0	0
POC	64	64	63‡	28	100 (94.3–100)	100 (87.7–100)	100 (90.0–100)	100 (87.7–100)	100 (90.0–100)	0	0
TE biopsy	52	52	52	19	98.1 (89.7–100)	94.7 (74.0–99.9)	100 (89.4–100)	100 (81.5–100)	97.1 (84.7–99.9)	0	2.9
Total	218	218	217	63	99.5 (97.5–100)	98.4 (91.5–100)	100 (97.6–100)	100 (94.2–100)	99.4 (96.5–100)	0	0.6

* CI denotes confidence interval, FDR false discovery rate, FNR false negative rate, NPV negative predictive value, POC products of conception, PPV positive predictive value, STORK short-read transpore rapid karyotyping, and TE trophectoderm.
† Cases of abnormality includes aneuploidy, large copy-number variants, and high-level mosaicism.
‡ One sample had no G-band result owing to culture failure.

short DNA fragments for aneuploidy testing of reproductive tissues supports the further investigation of STORK to guide prenatal and fertility care. Limitations of the assay include the inability to detect balanced translocations and some copy-number variants and types of polyploidy. However, the reduced cost, same-day turnaround time, ability to perform on-site testing without transporting the sample to a reference laboratory, ability to test for aneuploidy across all chromosomes, and elimination of the need for living tissue are key advantages of STORK that would be directly relevant to the vast majority of clinical cases (see the Discussion section in the Supplementary Appendix). Such benefits could reduce health care barriers and costs and thus lead to improved access to reproductive care.

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