CORRESPONDENCE

# Toward Same-Day Genome Sequencing in the Critical Care Setting

**TO THE EDITOR:** Rapid genetic diagnosis is increasingly used in critical care management, particularly in neonatal intensive care units (NICUs).[1-3] However, many critical care decisions occur on the scale of hours, whereas current options for clinically available rapid genomic sequencing take days (from sample receipt to report) at best. Scaling effective rapid diagnosis in critical care settings therefore hinges on workflows that compress preanalytic, analytic, and interpretive steps into same-day timeframes.

Although genome sequencing completed within hours has been shown to be attainable,[4,5] current methods are not amenable to a routine clinical offering. The key operational barrier is a lack of access to end-to-end sequencing workflows that maximize cost-effectiveness and speed and that can be run on demand with one or a few patient samples. Sequencing by expansion involves the synthesis of an expanded polymer with native DNA as a template. The polymer is then read by nanopore translocation.[6] The design of sequencing by expansion supports near-real-time data processing and adjustable run times and enables small-batch multiplexing. Integrated base calling, formation of a consensus read, and genome alignment are accelerated and concurrent with sequencing, producing a sorted binary alignment map nearly simultaneously with the end of sequencing. Variant calling (of single-nucleotide variants, indels [insertions and deletions], and copy-number variants) is also accelerated locally and begins immediately on the availability of completed binary-alignment-map files, generating a variant-call format from a binary alignment map in less than 30 minutes. We piloted this method in a cohort of infants in a NICU to investigate its feasibility and capacity to produce results that align with urgent critical care workflows.

Over the course of 3 weeks, we sequenced and analyzed 15 human genomes (1 or 2 per day) on the prototype of sequencing by expansion: 3 HG002 human reference samples obtained from the National Institute of Standards and Technology, 5 previously tested patient samples with known reportable results, and samples from a prospective set comprising 7 patients in the NICU (Fig. 1). A parent of each patient provided written informed consent to participate in the study, which was approved by the hospital's institutional review board. Parallel rapid testing was obtained from a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory for all prospective samples; all results obtained through sequencing by expansion were confirmed. The mean time to generate variants from genomic DNA was 4 hours 4 minutes (range, 3 hours 57 minutes to 4 hours 25 minutes). In the case of blood samples that arrived at the laboratory by 7 a.m., we obtained an interpreted report between 2 p.m. and 4:30 p.m. the same day. (Because these results were obtained during the course of research — unlike those obtained from the CLIA-certified laboratory — they were not used in making clinical diagnoses.) The fastest time from sample receipt to report was 6 hours 47 minutes.

The same-day results for the seven infants could have been actionable if they had been obtained as part of first-line clinical testing. However, no results were returned from this research study, and appropriate care plans were already in place in all cases. There were two positive findings and five negative findings. Infant 3 (who had multiple anomalies) had an unbalanced translocation between chromosomes 10 and 14. Infant 7 (who had tonic seizures) had a pathogenic variant in *KCNQ2*, which underlies a genetic epilepsy syndrome responsive to sodium-channel blockers. Findings in both infants were generated more quickly than the alternative currently available rapid genetic tests. Case details are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org. We have shown a scalable approach to same-day rapid genome

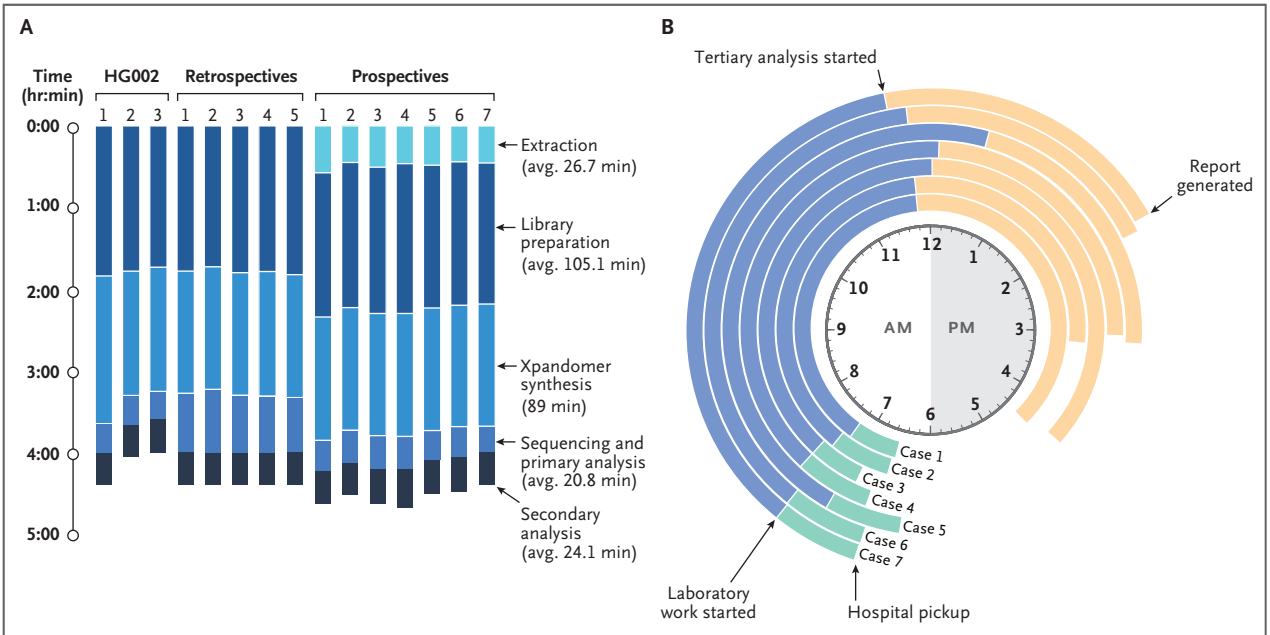


**Figure 1. Timing and Workflow for Sample Processing and Delivery.**

In Panel A, stacked bars indicate the durations of the laboratory, Xpandomer synthesis, sequencing and primary-analysis steps, and secondary-analysis steps, with the start time normalized to hour 0. HG002 comprises the three separate replicates of the National Institute of Standards and Technology reference sample that were processed on 3 separate days. The retrospectives samples comprise previously tested patient samples with known pathogenic variants. The prospectives samples comprise samples from the seven patients who were enrolled in the research study and whose samples were sent (one sample per day) to the laboratory for processing. The average duration in minutes (min) is shown for each step. The first run of HG002 had a longer synthesis time; all other samples were fixed at 89 minutes. In Panel B, radial plot bars indicate the wall-clock timing for each of the prospective cases, including transport time (green), laboratory processing and sequencing (blue), and time for tertiary analysis and report generation (yellow).

sequencing in a NICU setting using a novel, in-development platform in a timeframe that would support rapid clinical decision making.

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