HYRAX BIOSCIENCES

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Analytical Validation of the Thermo Fisher ABI HIV-1 Genotyping Kit for Oxford Nanopore Sequencing Against the Sanger Gold Standard Using the Exatype Platform

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Executive Summary

Exatype, a cloud-based DNA-sequence analysis platform, developed by Hyrax Biosciences, underpins **HIV drug-resistance testing** for programmes worldwide, supporting more than **1,100 users** across **70+ organisations** in **103 countries** and processing over **150,000 HIV sequences** to date. Its broad adoption - particularly across Global Health Equity settings - has established Exatype as a trusted platform for routine Sanger-based **HIV-1 genotyping and reporting**.

Given this widespread adoption, Hyrax Biosciences evaluated whether data generated using the Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase, when sequenced on Oxford Nanopore Technologies (ONT), delivers **drug-resistance results** equivalent to those generated from Sanger sequencing, with both datasets analysed using the Exatype platform. These findings show that the assay **performs consistently** across both technologies, with **strong concordance in consensus sequences and drug-resistance calls.** Exatype served as the **shared analytical workflow**, **enabling direct comparison** and **simplifying cross-platform interpretation**.

Key outcomes:

- **Specificity and repeatability**: 100% concordance with Sanger and between ONT replicates.
- **Sensitivity**: Consistent detection of resistance mutations at ≥20% prevalence.
- **Native vs Rapid ONT workflows validated:** both equivalent to Sanger, with trade-offs in depth (Native) and speed (Rapid).
- **Exatype-driven standardisation:** A unified analytical layer enabling QC, consensus generation, and HIVDB reporting across sequencing technologies.

Background and Rationale

As HIV antiretroviral therapy coverage expands - reaching **29.8 million people** by 2022 (WHO, 2025)[1] - selective pressure has increased the emergence of **HIV drug resistance**, driving demand for **scalable, reliable genotyping** (WHO, HIV Drug Resistance)[2]. With laboratories

now incorporating flexible platforms such as ONT alongside traditional Sanger sequencing, a **single, standardised workflow** becomes increasingly valuable.

This study **validates** the use of the Applied Biosystems HIV-1 Genotyping Kit with Integrase from Thermo Fisher Scientific, sequenced with ONT and benchmarked against Sanger. Both datasets were analysed through Hyrax Biosciences' Exatype platform, providing a like-for-like comparison and confirming that the assay can be deployed reliably across **multiple sequencing technologies.**

Validation Results

Study design: included 2 samples × 3 replicates for protease (PR) and reverse transcriptase (RT) - as a single amplicon - and integrase (IN) amplified with Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase, sequenced on ONT and Sanger, and analysed using Exatype. Two separate barcoding strategies were tested on the ONT platform, the standard long-read barcoding (termed **Native**) and a short-read barcoding (termed **Rapid**).

Quantitative Outcomes

ONT (Native and Rapid) vs Sanger

Metric	Result	Benchmark / Notes
Specificity	100% concordance between ONT and Sanger consensus sequences	Validates assay equivalence
Repeatability	100% concordance across replicates	Demonstrates assay robustness
Sensitivity	Matching variant detection at ≥20% threshold (industry standard)	Confirms equivalence with Sanger
Sensitivity threshold	Sanger threshold 15 - 20%[3]; ONT threshold ≥1%	Improved detection of minor variants with ONT
Drug-resistance calls	Consistent across Sanger and ONT	Reliable HIV-DR detection

Native vs Rapid ONT Strategies

Metric	Native ONT	Rapid ONT	Benchmark / Notes
Average Read Length	1,042 nt	353 nt	Both read lengths are sufficient for HIV-DR genotyping, demonstrating ONT's versatility
Average Quality Score	Q=28	Q=24	Quality exceeded the ONT minimum (Q=8)

Average Read Depth / Coverage across *pol*	111k / 80k	37k / 12k	Both datasets exceed the minimum coverage (15 reads) needed to reliably detect variants present at ≥20%*
Average Runtime on Exatype	21 min	5 min	Shorter runtime for shorter read lengths (Rapid ONT)
Concordance with Sanger	100%	100%	Consensus sequences were concordant despite variation in sequencing metrics

Key Takeaways:

- **Specificity:** ONT (Native & Rapid) matches Sanger; identical consensus sequences and HIV-1 drug-resistance calls.
- **Sensitivity:** Detects variants at ≥20% prevalence; ONT also captures lower-prevalence variants.
- **Speed vs Coverage:** Native = longer reads, higher depth; Rapid = faster analysis with shorter reads.
- **Practical Impact:** Both strategies support reliable, high-throughput HIV-DR testing.

*Sequencing depth of the Rapid ONT dataset may not represent all rapid-barcoding strategies.

Analytical and Applied Implications enabled by Hyrax Biosciences' Exatype Platform

This validation demonstrates that the Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase **performs consistently** when sequenced on ONT, producing **drug-resistance results** that align with those obtained from Sanger sequencing. Exatype served as the **shared analytical environment**, applying the same interpretation rules, thresholds, and reporting structure across both datasets to enable a **direct, like-for-like comparison of assay performance**.

Exatype retains compatibility with ≥20% reporting thresholds while also accommodating the richer variant signal available from ONT, supporting programmes moving toward higher-throughput or decentralised sequencing. Automated QC, consensus generation, and standardised reporting **remove the need for platform-specific bioinformatics**, enabling laboratories to increase capacity and adopt ONT without changing analytical workflows.

Conclusion

This validation confirms that data generated using the Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase on ONT, when analysed through Exatype, produces HIV-1 drug-resistance results equivalent to Sanger. Both Native and Rapid ONT workflows demonstrate reliable

analytical performance while offering the operational flexibility associated with decentralised sequencing.

Together, these findings position **Exatype** as a consistent, **cross-platform analytical solution** that supports ONT adoption in routine testing, surveillance, and decentralised HIV-DR applications while preserving full comparability with established Sanger workflows.

References

1. World Health Organisation (WHO). *HIV statistics, globally and by WHO region*, Information Sheet, 2025
2. World Health Organisation (WHO), *HIV drug resistance*
3. Steegan *et al.*, 2023, *Advancing HIV Drug Resistance Technologies and Strategies: Insights from South Africa's Experience and Future Directions for Resource-Limited Settings*, Diagnostics, 13(13):2209