Unveiling the Diagnostic Potential: A Multi-Center Evaluation of the RPIP Target Capture Sequencing Kit for Pathogen Detection, AMR Profiling and Strain Typing from Common Respiratory Specimens

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Introduction

The Illumina Explify RPIP is a target capture sequencing (TCS) assay capable of detecting over 280 respiratory pathogens and their associated AMR determinants [1].

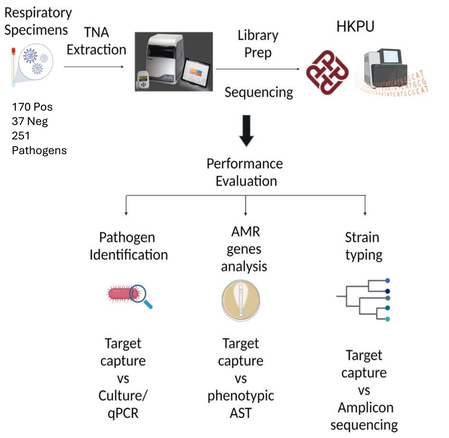
The Knowledge Gap:

Previous studies were limited to single-center cohorts and single specimen types (e.g., BAL fluid) and focused primarily on pathogen identification [2]. A comprehensive, multi-center validation across common respiratory specimens for simultaneous pathogen ID, AMR prediction, and viral lineage profiling **is missing**.

Study Aim:

1. **Benchmark** RPIP Pathogen ID across diverse respiratory specimens.
2. **Evaluate performance** in MDRO phenotype and drug susceptibility prediction.
3. **Assign Lineages** for priority viral pathogens, including SARS-CoV-2 and Influenza A.

Methodology



Sample Cohort: 207 leftover respiratory specimens (Sputum, swabs, BAL, aspirates) with known culture or qPCR results, collected across **5 public hospitals**

Pipeline Benchmarking: Two GUI bioinformatics tools, (**DME+** [1], **CZID** [3]) and one in-house pipeline (**KrakenUniq** [4]) were benchmarked for Pathogen ID. **DME+**, **CZID** and **Resfinder** [5] were benchmarked for AMR detection.

Pathogen ID Evaluation: RPIP workflows were compared directly against **conventional culture** and **qPCR**

ARG Detection Evaluation: 35 specimens containing **40 MDROs** were analyzed and compared to **phenotypic DST profiles.**

Viral Lineage Assignment Evaluation: 15 SARS-CoV-2 and **24 Influenza A** samples, evaluated against the wet-lab **ONT Midnight** and **Influenza Whole-Genome Sequencing** protocols, respectively.

Discussion

Pipeline Standardization: The **lack of consensus guidelines** and operational cut-off thresholds for bioinformatics tools could significantly impacts diagnostic performance, presenting a major hurdle for clinical implementation.

Specimen Preference: Sputum and lower respiratory tract specimens remain the **preferred specimen types** to ensure optimal diagnostic yield.

Genotype-Phenotype Discrepancies: TCS reliably determines overall MDRO status from direct, but drug-specific predictions vary. The observation of both ARG-positive/pDST-sensitive strains and ARG-negative/pDST-resistant strains demonstrates that the presence of an ARG targeting a specific drug class **does not perfectly dictate phenotypic outcomes**, highlighting a critical need for continuous genotype-phenotype mapping.

Conclusion

Unified Pipeline: Streamlines simultaneous pathogen detection, AMR profiling, and viral strain typing into a **single multi-center framework.**

High Clinical Accuracy: Yielded **82.3%** pathogen detection accuracy in sputum and an **86%** genotypic concordance rate to phenotypic AST.

Robust Surveillance: Secured **identical lineage assignments** for priority respiratory viruses (SARS-CoV-2 and Influenza A) against long-read ONT workflows.

Result

1. Evaluation of Diagnostic Performance using Three Distinct Bioinformatics Tools

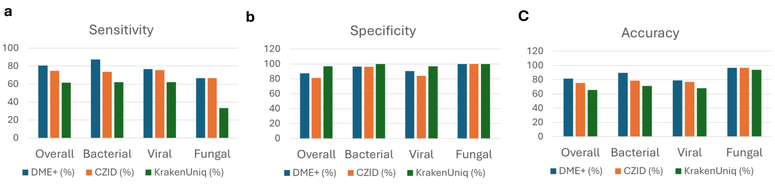


Figure 1. Pathogen-specific diagnostic performance of three bioinformatics tools. (a) Sensitivity, (b) specificity, and (c) overall accuracy comparison of the DME+, CZID, and KrakenUniq pipelines across overall, bacterial, viral, and fungal classifications.

2. Diagnostic Performance Across Specimen Types

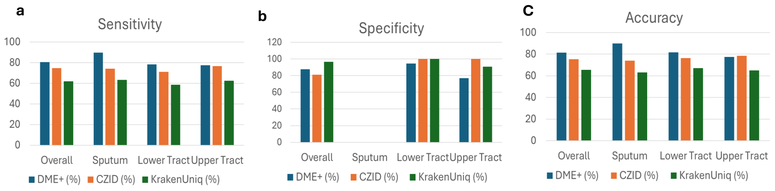


Figure 2. Comparison of diagnostic metrics by respiratory specimen type. (a) Sensitivity, **(b)** specificity, and **(c)** accuracy evaluation across different specimen types (sputum, lower respiratory tract, and upper respiratory tract specimens).

3. MDRO Genotype Detection and Susceptibility Prediction

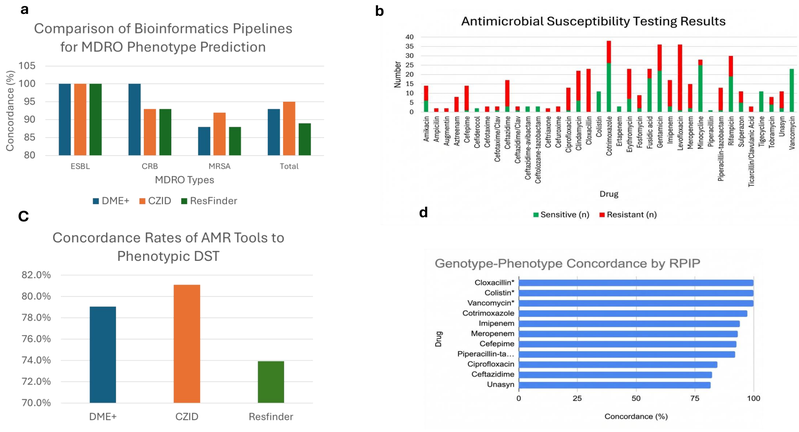


Figure 3. MDRO Genotype detection and drug susceptibility prediction. (a) Performance comparison of DME+, CZID, and ResFinder pipelines across major MDRO categories. **(b)** Summary of phenotypic DST results showing sensitive vs. resistant strain counts. **(c)** Overall concordance of AMR tools relative to phenotypic DST result. **(d)** Selected drug-level genotype-phenotype mapping with good concordance. Asterisks (*) indicate drugs where all tested isolates were either entirely sensitive or entirely resistant.

4. SARS-CoV-2 and Influenza A Genomic Characterization

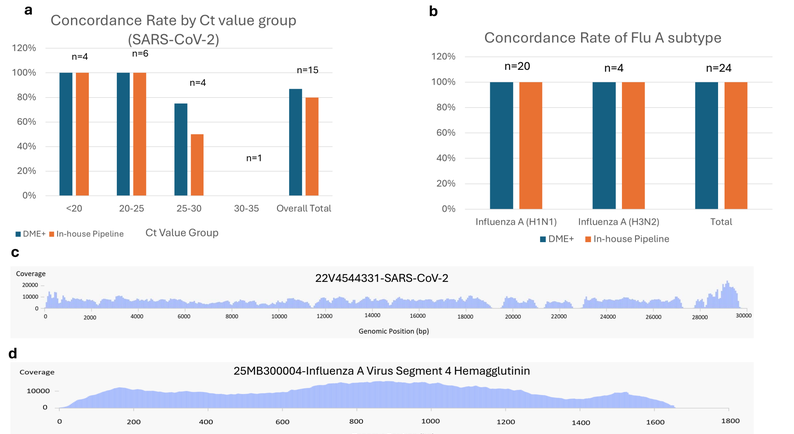


Figure 4. RPIP viral lineage assignment concordance rates. (a) SARS-CoV-2 lineage concordance stratified across progressive cycle threshold (Ct) value groups. **(b)** Influenza A concordance categorized by viral subtype (H1N1 and H3N2).**(c)** Genome coverage plot of a representative sample with SARS-CoV-2 Coverage depth =6884X; Coverage breadth=97.1% **(d)** Genome coverage plot of a representative sample with Influenza A. Coverage depth =9303X; Coverage breadth=94.5%

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Reference

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