Explify® Enrichment
▶Respiratory ID/AMR Panel

ACCESSION: **RPIP-Example-Full-Feature-Demo**
REPORT DATE: **24 October 2022**

DATE OF BIRTH: **Not Provided**
SEX: **Not Provided**
EXPLIFY ID: **57be9f71c512002**
PRESENTATION VERSION: **2.0.0**

SUBMITTER: **Not Provided**
ANALYSIS PIPELINE VERSION: **4.25.0**

SAMPLE RECEIVED: **24 October 2022**
DATE OF COLLECTION: **Not Provided**
SAMPLE TYPE: **Not Provided**
TEST VERSION: **5.11.3**

Analysis Performed: Explify® Respiratory Pathogen ID/AMR Panel (RPIP) - Data Analysis Solution
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RESULTS: ONE OR MORE POTENTIAL PATHOGENS DETECTED

BACTERIA

	QUANTITY (PROPORTION OF DETECTED BACTERIA)[1]	ASSOCIATED AMR MARKER DETECTED[2]	PHENOTYPIC GROUP[3]
Klebsiella pneumoniae **Potential ESBL**	6.0 x 10^5 copies/mL (71.4%)	**Yes**	2
Mycobacterium tuberculosis complex	2.4 x 10^5 copies/mL (28.5%)	**Yes**	3

VIRUSES

	QUANTITY (PROPORTION OF DETECTED VIRUSES)[1]	POTENTIAL AMR DETECTED[2]	PHENOTYPIC GROUP[3]
Influenza A virus (H1N1) **Potential NAI Resistance**	1.2 x 10^6 copies/mL (77.0%)	**Yes**	3
SARS-CoV-2 (2019-nCoV) **BA.5.1**[4]	3.6 x 10^5 copies/mL (22.9%)	n/a	3

FUNGI

	QUANTITY (PROPORTION OF DETECTED FUNGI)[1]	POTENTIAL AMR DETECTED[2]	PHENOTYPIC GROUP[3]
Cryptococcus neoformans	1.2 x 10^5 copies/mL (100%)	n/a	3

AMR

	DRUG CLASS[5]	ASSOCIATED MICROORGANISMS DETECTED[6]
CTX-M (Best Match: CTX-M-94) **ESBL**	Cephalosporin (1st Generation) Cephalosporin (3rd Generation) Cephalosporin (4th Generation) Penicillin	Klebsiella pneumoniae
embB (Variants: F330S, Y333H)	Polyamine Antibiotic Rifamycin Antibiotic	Mycobacterium tuberculosis complex
embC (Variants: I297L+W326R[7])	Polyamine Antibiotic	Mycobacterium tuberculosis complex
NA (Variants: H275Y)	Neuraminidase inhibitor	Influenza A virus (H1N1)

Footnotes

1. Absolute quantification assumes use of Enterobacteria phage T7 as an Internal Control spiked at 1.21 x 10^7 copies/mL of sample. Relative abundance is calculated based on absolute quantities and is expressed as proportion of absolute quantities within each pathogen class (i.e., bacteria, viruses, fungi). If RPKM for the Internal Control is zero, no absolute quantification is provided, and relative abundance is expressed as proportion of microorganism RPKM values within each pathogen class (i.e., bacteria, viruses, fungi).

2. The Explify RPIP Data Analysis Solution predicts resistance of 79 respiratory pathogens to 26 relevant drug classes based on detection of 2,097 associated antimicrobial resistance (AMR) markers unless filtered reporting options are selected. Detection of an associated AMR marker is reported if the AMR marker passes a minimum detection threshold and if one of the respiratory pathogens associated with the AMR marker is also detected, in alignment with guidance provided by the College of American Pathologists (CAP) MIC.21855 (new 09/22/2021). Association between respiratory pathogen and AMR marker is based on scientific literature and the Comprehensive Antibiotic Research Database Prevalence Data (CARD Prevalence, version 3.0.9) from McMaster University. Reported AMR markers have been associated with antimicrobial resistance but may not always indicate phenotypic resistance. Failure to detect AMR markers does not always indicate phenotypic susceptibility. Results should be interpreted in the context of all available information.

3. Targeted microorganisms are classified into three Phenotypic Groups based on general association with normal flora, colonization, or contamination from the environment or other sources, as well as based on general association with disease. Phenotypic grouping DOES NOT INDICATE PATHOGENICITY IN A GIVEN CASE and results need to be interpreted in the context of all available information. Phenotypic Group 1: Microorganisms that are frequently considered part of the normal flora, colonizers, or contaminants but may be associated with disease in certain settings. Phenotypic Group 2: Microorganisms that may represent normal flora, colonizers, or contaminants but that are frequently associated with disease. Phenotypic Group 3: Microorganisms that are not generally considered part of the normal flora, colonizers, or contaminants and are generally considered to be associated with disease.

4. The most likely Pango (phylogenetic assignment of named global outbreak) lineage is assigned to the majority consensus SARS-CoV-2 (2019-nCoV) genome sequence using pangolin v4.1.3 (Áine O'Toole & Emily Scher et al. 2021 Virus Evolution DOI:10.1093/ve/veab064).

5. Detected AMR markers may not confer resistance to every antimicrobial in the drug class and may also confer resistance to drug classes that are not listed. Linkage between bacterial AMR marker and drug class is based on the Comprehensive Antibiotic Research Database (CARD, version 3.1.0) from McMaster University, ResFinder (version 2021-01-20), NCBI Reference Gene Catalog (version 2020-12-17.1), EUCAST expert rules on indicator agents (2019/2020), and CLSI Performance Standards for Antimicrobial Susceptibility Testing (M100 31st Edition). Linkage between viral AMR marker and drug class is based on the publications provided in the JSON report - see PubMed IDs (pmids) field.

6. A representative list of associated microorganisms known to harbor the detected or similar bacterial AMR markers, based on the Comprehensive Antibiotic Research Database Prevalence Data (CARD Prevalence, version 3.0.9) from McMaster University, can be found in the Commonly Associated Microorganisms field.

7. Mutations connected with a '+' form an epistatic group. Epistatic groups are two or more mutations that need to be present concurrently to confer the associated resistance.

Abbreviations

AMR (antimicrobial resistance); ESBL (extended spectrum beta-lactamase); mL (milliliter); NAI (neuraminidase inhibitor); NGS (next-generation sequencing); PAI (polymerase acidic endonuclease inhibitor); pangolin (phylogenetic assignment of named global outbreak lineages); RPIP (Respiratory Pathogen ID/AMR Panel); RPKM (Reads Per Kilobase of target per Million mapped reads)

ADDITIONAL INFORMATION

READ CLASSIFICATION[9]: **93.8% Targeted** **5.7% Untargeted** **0.5% Ambiguous** **0.0% Unclassified**

The following tables represent additional information and/or different presentation from what is shown above.

Table 1 lists frequently used antibiotic classes (“Drug Class”), whether potential AMR markers were detected for the respective Drug Class, known intrinsic resistance, and the detected AMR marker with the best matching allele in the reference sequence database or the detected variant.

Table 1. Antibiotic drug classes, known intrinsic resistance, and detected AMR markers

DRUG CLASS	INTRINSIC RESISTANCE OR POTENTIAL AMR MARKER DETECTED	INTRINSICALLY RESISTANT DETECTED MICROORGANISM[10]	AMR MARKER (BEST MATCH ALLELE / DETECTED VARIANT)
Aminoglycoside	No	-	-
Beta-Lactam + Beta-Lactamase Inhibitor	No	-	-
Carbapenem	No	-	-
Cephalosporin (1st Generation)	**Yes**	-	CTX-M(CTX-M-94)
Cephalosporin (2nd Generation)	No	-	-
Cephalosporin (3rd Generation)	**Yes**	-	CTX-M(CTX-M-94)
Cephalosporin (4th Generation)	**Yes**	-	CTX-M(CTX-M-94)
Cephalosporin (Unknown)	No	-	-
Diaminopyrimidine	No	-	-
Fluoroquinolone	No	-	-
Fosfomycin	No	-	-
Glycopeptide	**Yes**	Klebsiella pneumoniae	Intrinsic Resistance
Lincosamide	**Yes**	Klebsiella pneumoniae	Intrinsic Resistance
Macrolide	**Yes**	Klebsiella pneumoniae	Intrinsic Resistance
Oxazolidinone	**Yes**	Klebsiella pneumoniae	Intrinsic Resistance
Penicillin	**Yes**	Klebsiella pneumoniae	Intrinsic Resistance CTX-M(CTX-M-94)
Polymyxin	No	-	-
Sulfonamide	No	-	-
Tetracycline	No	-	-

Table 2 lists frequently used antitubercular drug classes (“Drug Class”), whether potential AMR markers were detected for the respective Drug Class, and the detected AMR marker with the best matching allele in the reference sequence database or the detected variant.

Table 2. Antitubercular drug classes and detected AMR markers. The reported AMR marker variants and representative antitubercular drugs apply only to Mycobacterium tuberculosis complex.

	DRUG CLASS	POTENTIAL AMR MARKER DETECTED	AMR MARKER (BEST MATCH ALLELE / DETECTED VARIANT)
First-Line	Isoniazid	No	-
	Polyamine Antibiotic	**Yes**	embB(F330S), embC(I297L+W326R[7])
	Pyrazinamide	No	-
	Rifamycin Antibiotic	**Yes**	embB(Y333H)
Second-Line	Aminoglycoside	No	-
	Ethionamide	No	-
	Fluoroquinolone	No	-
	Para-Aminosalicylic Acid	No	-

Table 3 lists frequently used antiviral drug classes (“Drug Class”), whether potential AMR markers were detected for the respective Drug Class, and the detected AMR marker with the detected variant.

Table 3. Antiviral drug classes and detected AMR markers

DRUG CLASS	POTENTIAL AMR MARKER DETECTED	AMR MARKER (DETECTED VARIANT)
Endonuclease Inhibitor	No	-
Neuraminidase Inhibitor	**Yes**	NA(H275Y)

Table 4 lists microorganisms commonly associated with a detected AMR marker. Commonly associated microorganisms include microorganisms that were NOT detected but that are associated with the detected or similar AMR markers.

Table 4. AMR Markers and Commonly Associated Microorganisms

DETECTED AMR MARKER	CONFIDENCE[11]	COMMONLY ASSOCIATED MICROORGANISMS[12]
CTX-M (Best Match: CTX-M-94) **ESBL**	High	Acinetobacter baumannii Aeromonas caviae Aeromonas hydrophila Aeromonas veronii Citrobacter freundii complex Citrobacter koseri Enterobacter cloacae complex Escherichia coli Klebsiella oxytoca Klebsiella pneumoniae Klebsiella quasipneumoniae Leclercia adecarboxylata Morganella morganii Proteus mirabilis Providencia stuartii Pseudomonas aeruginosa Raoultella planticola Salmonella enterica Serratia marcescens
embB (Variants: F330S, Y333H)	High	Mycobacterium tuberculosis complex
embC (Variants: I297L+W326R[7])	High	Mycobacterium tuberculosis complex

Footnotes

8. Footnote intentionally left blank.

9. This test differentiates sequencing reads classified to microorganism genomic regions that were targeted by capture probes (“Targeted”) from those that are not targeted (“Untargeted”), cannot be unambiguously assigned to one category (“Ambiguous”), or cannot be classified with confidence (“Unclassified”).

10. All intrinsic resistance described in CLSI Performance Standards for Antimicrobial Susceptibility Testing, M100 31st Edition, Appendix B for detected microorganism(s) is reported for listed drug classes; however, detected microorganism(s) may not be intrinsically resistant to every antimicrobial in the drug class. Additional comments regarding CLSI intrinsic resistance definitions may be reported in footnotes specific to the detected microorganism(s).

11. Confidence of bacterial AMR marker detection is shown as High or Medium and is based on the available sequencing data. High confidence indicates that a bacterial AMR marker passes the detection threshold defined by the Comprehensive Antibiotic Research Database (CARD) from McMaster University (blastP bitscore) or has 100% sequence coverage and percent protein sequence identity (PID). Medium confidence indicates that a bacterial AMR marker does not pass the CARD detection threshold, but does have ≥80% sequence coverage.

12. Detected AMR markers are reported if one or more associated microorganisms are detected and reported, in alignment with guidance provided by the College of American Pathologists (CAP) MIC.21855 (new 09/22/2021). However, detected bacterial AMR markers may originate from microorganisms that did not meet detection thresholds or microorganisms not targeted by the test.

INTERPRETIVE DATA

For Research Use Only. Not for use in diagnostic procedures.

The Explify RPIP Data Analysis Solution identifies 42 viruses, 187 bacteria, 53 fungi, and 2,097 AMR markers, unless filtered reporting options are selected, based on target enriched next-generation sequencing (NGS) of microorganism transcriptome and genome sequences. Sequencing data are interpreted by the Explify software platform and microorganisms that pass detection thresholds are reported. Absolute quantification assumes use of Enterobacteria phage T7 as an Internal Control spiked at 1.21 x 10^7 copies/mL of sample. Relative abundance is calculated based on absolute quantities and is expressed as proportion of absolute quantities within each pathogen class (i.e., bacteria, viruses, fungi). If RPKM for the Internal Control is zero, no absolute quantification is provided, and relative abundance is expressed as proportion of microorganism RPKM values within each pathogen class (i.e., bacteria, viruses, fungi).

This test predicts resistance of 79 respiratory pathogens to 26 relevant drug classes unless filtered reporting options are selected. Detection of a respiratory pathogen known to harbor the detected AMR marker is required for reporting, in alignment with guidance provided by the College of American Pathologists (CAP) MIC.21855 (new 09/22/2021). Detection of AMR markers, including mutations, does not always indicate that a microorganism is phenotypically resistant. Failure to detect AMR markers, including mutations, does not always indicate phenotypic susceptibility. Results should be interpreted in the context of all available information.

See https://www.illumina.com/ for additional information.

LIMITATIONS

Non-detected results do not rule out the presence of viruses, bacteria, fungi, and AMR markers. Contamination with microorganisms is possible during specimen collection, transport, and processing. Closely related microorganisms may be misidentified based on sequence homology to species present in the database. The identification of cDNA or DNA sequences from a microorganism does not confirm that the identified microorganism is causing symptoms, is viable, or is infectious. Recombinant viral strains may not be reported or may be reported as one or more individual viruses. Should one or more individual viruses be reported for a recombinant viral strain, antiviral resistance results may be inaccurate.

Detection of AMR markers does not always predict phenotypic resistance; lack of detection does not indicate susceptibility. The best matching allele is reported for each detected AMR gene family. In bacterial strains harboring two or more alleles within the same AMR gene family, only the allele with the higher confidence will be reported as the best match. In strains containing insertion-deletion mutations (indels), there is a risk of false positive or false negative results for other resistance mutations within a region of 100 nucleotides around the indel.

Information provided by the Explify RPIP Data Analysis Solution is based on scientific knowledge and has been curated; however, scientific knowledge evolves and information about associated microorganism and associated resistance may not always be complete and/or correct. Results should be interpreted in the context of all available information. Other sources of data may be required for confirmation.