**Methods**

Sample Collection and Extraction

Between 200-500 mL of raw wastewater is collected by fourteen participating Wastewater Treatment Facilities from five cities in Texas. Samples are collected in 500 mL leak-proof prelabeled bottles, decontaminated, and sealed into biohazard bags, then shipped overnight with ice packs to the Alkek Center of Metagenomics and Microbiome Research at Baylor College of Medicine, Houston, TX. Sample bottles are barcoded upon arrival and refrigerated until they undergo filtration and DNA/RNA extraction using Qiagen Qiamp VIRAL RNA Mini Kit.

Quantitative Polymerase Chain Reaction (PCR)

Real-time RT-PCR for select targeted viruses is performed using the TaqPath 1-step Multiplex Master Mix (A28523 Applied Biosystems) on the 7500 Fast Dx Real-Time PCR Instrument ([4406985](https://www.hermofisher.com/order/catalog/product/4406985), Applied Biosystems) with SDS version 1.4 software. Samples are considered positive if Ct values are less than 45. The real-time RT-PCR protocol includes negative extraction and no template controls. A standard curve with amplicons targeting the primers and probe sequences of each target is used to determine the genomic copy numbers used in downstream analysis.

Comprehensive Deep Sequencing (CDS)

RNA extracts are converted to cDNA and a probe-based viral capture is performed on the DNA/cDNA mix using the Twist Comprehensive Viral Research Panel (Twist Biosciences). Sequencing library construction is performed using the Twist Library Preparation EF 2.0 Kit and Twist Universal Adaptor System. Samples are then sequenced on an Illumina NovaSeq 6000 SP flow cell, to generate 2×150 bp paired-end reads.

Bioinformatic Data Processing of CDS

A custom mapping and taxonomic classification pipeline and Virus Database have been developed for processing of the comprehensive deep sequencing data. The pipeline generates per sample abundance and coverage metrics and taxonomic information used to create the visualizations in this report. The Virus Database is composed of a dereplicated set of reference genomes downloaded from NCBI that encompass the species and strains covered by the Twist Comprehensive Virus Research Panel capture probe panel. Additionally, 97 viral pathogens of particular public health interest were identified, their RefSeq genomes downloaded and included in the database, independently of the prior dereplication.

For a more detailed explanation of the methods used to generate and analyze the data please see: Tisza and Javornik Cregeen et al., “Comprehensive Wastewater Sequencing Reveals Community and Variant Dynamics of the Collective Human Virome” [in submission; biorxiv doi placeholder].