HIV Detection in Wastewater as a New Epidemiological Tool

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BACKGROUND

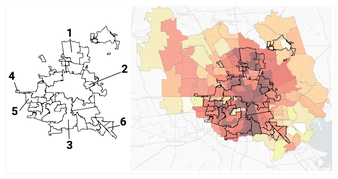
- Wastewater (sewage) represents a broad, immediate, and unbiased accounting of pathogens in the population.
- We aimed to develop methods to track HIV in wastewater utilizing a viral detection pipeline adapted from platforms developed to track SARS-COV-2.

METHODS

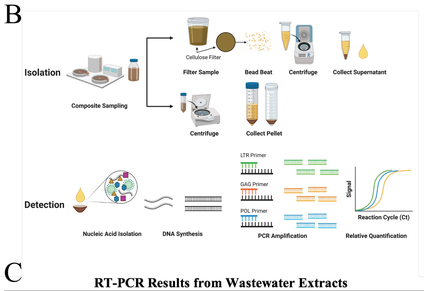
- We used samples from 6 wastewater treatment plants in the Houston area. We focused on regions of higher prevalence and lower prevalence (**Fig 1A**).
- Employing wastewater processing and nucleic acid extraction methods described by our group to detect SARS-COV-2 (**Fig 1B**), we tested a single high and low prevalence site in triplicate with 3 primer sets targeting the *gag*, *pol* and the LTR regions.
- Once these techniques were refined, subsequent samples with RT-PCR positive results were subjected to gel electrophoresis and genetic sequencing.
- In a later set of studies, we fractionated samples into supernatant and pellet. HIV detection in wastewater was confirmed by performing whole virome sequencing.

Figure 1

A



B



C

RT-PCR Results from Wastewater Extracts

	Melting Temperature (°C)			Cycle Threshold		
Sample	**LTR**	**GAG**	**POL**	**LTR**	**GAG**	**POL**
HIV	83.8	79.2	78.8	20.3	16.1	18.6
HIV Spike				20.5	15.5	16.8
SIV	74.8	74.3	71.9	34	29.2	33.8
Site #2 (Higher Prevalence)	74.6	80.4	78.2	NA	26.8	27.2
Site #4 (Lower Prevalence)	74.9	80.3	74.7	NA	28.5	27.8

Fig. 1. Wastewater sample selection, processing, and analysis for HIV detection.

(A) The wastewater treatment plants selected for this study on left, based on overlay onto the AIDSVu map for Houston on right, where HIV prevalence is reported by zip code (yellow/light=lower prevalence, red/dark=higher prevalence).

(B) Wastewater processing and detection schematic. Samples were processed by either electronegative filter concentration or pelleting. Nucleic acid was extracted from these samples, then subjected to RT-PCR by either SYBR green or probe methods to detect HIV.

(C) Results of pilot study, where *gag* and *pol* primers produced products with melting temperatures consistent with HIV detection (bolded cells). HIV spike was 1µL extracted HIV RNA added to 1µL extracted nucleic acid from Site #2 prior to detection by RT-PCR. Numbers reported are averages of 3 technical replicates.

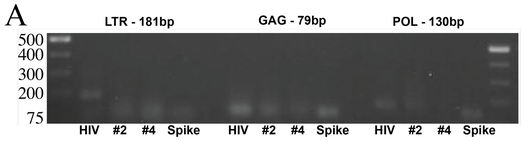
HIV can be detected in municipal wastewater. Wastewater detection of HIV merits further development as a new epidemiologic tool in ending the HIV epidemic.

RESULTS

- Samples from all sites resulted in signal detection at least once over time.
- Only PCR reactions with *gag* and *pol* primers appeared to amplify the expected product (**Fig 1C, 2A**).
- Products from a control spiked with HIV mapped almost exclusively to the HIV genome (97-100% of reads), while a control amplifying SIV genes yielded fewer matches (16-18% of reads) as expected.
- LTR and *pol* products did not map to the HIV genome; *gag* products did (34-44% of reads; **Fig 2B**).
- Among the fractionated samples, in total, 0 of 6 supernatant fractions produced detection compared to 7 of 8 pellet fractions (**Fig 3A**).
- The whole virome sequencing of nucleic acids from pellets produced reads that mapped to the HIV genome with at least 8X depth coverage. The sample with the lowest Ct detection (26) yielded HIV coverage several logs greater than samples with higher Ct detection (37; **Fig 3B**).
- Reads from all samples mapped to at least 20% of the HIV genome (**Fig 3C**).

Figure 2

A



B

Sequencing of RT-PCR Products

Primer pair	LTR				GAG				POL			
Sample	HIV (+)	SIV (-)	Site #2	Site #4	HIV (+)	SIV (-)	Site #2	Site #4	HIV (+)	SIV (-)	Site #2	Site #4
# of reads	8,316	2,008	2,186	2,812	11,788	4,544	2,858	2,816	6,652	4,408	7,664	11,344
# of mapped reads	8,037	350	120	262	11,731	734	1,253	944	6,605	814	4	2
% of mapped reads	97	17	5	9	100	16	44	34	99	18	0	0

Fig. 2. Characterization and sequencing of RT-PCR products from wastewater extracts.

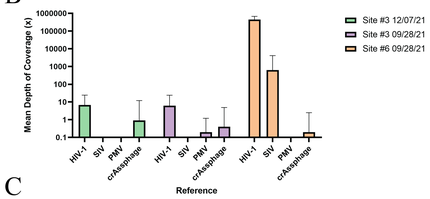
(A) Gel electrophoresis of RT-PCR products amplified with LTR (left), *gag* (middle), and *pol* (right) primers. Ruler on left denotes base pair length, with expected size listed next to primer name. Each set contained products from HIV RNA extract, nucleic acid extract from Sites #2 and #4, and HIV RNA extract added to Site #2 extract. (B) RT-PCR products from reactions with HIV, SIV, and Sites #2 and #4 extracts were sequenced and reads mapped to the expected product for each primer set. The number of reads and percentage of reads mapped to expected product are reported.

Figure 3

A

Collection Date	Samples with Detection	Method	Ct Values	Fraction	Replicates	Detections
6/1/21	Site #2-5	SYBR	26-29			
6/22/21	Site #1-6	SYBR/Probe	23-29			
8/17/21	Site #3,5	Probe	43, 40			
9/28/21	Site #3,6	Probe*	26-38	Supernatant	2	0
				Pellet	2	2
12/7/21	Site #3	Probe*	37	Supernatant	1	0
				Pellet	1	1
5/17/22	Site #3	Probe	34	Supernatant	3	0
				Pellet	5	4

B



C

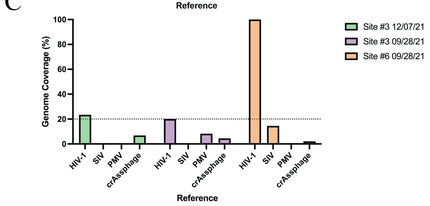


Fig. 3. Detection of HIV nucleic acid by RT-PCR and virome sequencing.

(A) Summary of collection dates, sites, and methods yielding detections by RT-PCR. Asterisk (*) denotes extracts submitted for whole virome sequencing.

(B) Mean depth coverage was determined by separately mapping paired short-reads to references (X-axis) with Geneious Assembler. Error bars represent the standard deviation of depth coverage across the entire reference.

(C) Genome coverage was calculated from read mapping described in panel B by determining the number of nucleotides aligned to at least one read compared to the number of nucleotides in the reference. References used were HIV-1 (NC_001802.1), SIV (NC_001549.1), PMV (NC_003630.1), and crAssphage (NC_024711.1). PMV, pepper mild mottle virus.

CONCLUSIONS

- HIV is detectable in wastewater, including detection of 100% of the genome in some samples.
- This work provides the first evidence that HIV can be detected in municipal wastewater systems.
- In this pilot work, DNA was detected but not RNA, and the signal was in the solid but not the liquid compartment, suggesting that the HIV is cellular in origin.
- Wastewater monitoring as a new tool in understanding the epidemiology of HIV in near real-time merits further research and development.

ADDITIONAL KEY INFORMATION

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