Metagenomics and Metadesign of the Subways and Urban Biomes Conference
Miami, November 2022

Utility of wastewater-based epidemiology to detect multiple human pathogens

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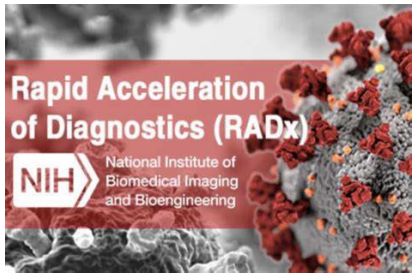

SYLVESTER COMPREHENSIVE CANCER CENTER
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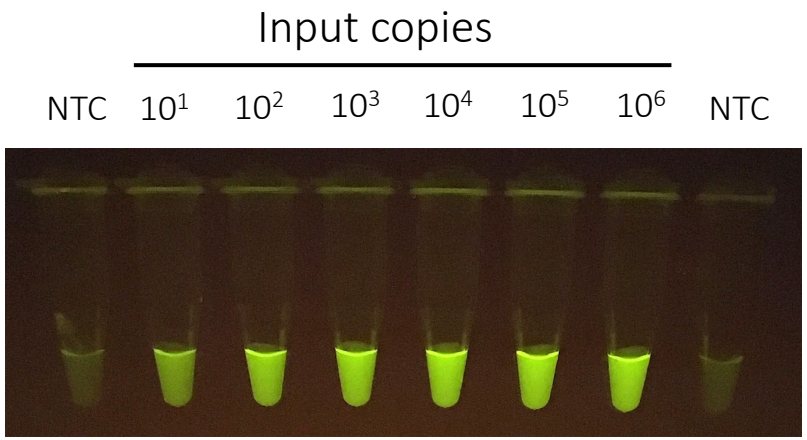
Development of PCR assay to detect SARS-CoV-2

- Ideally would be rapid, cost-effective and high-throughput
- Efficient with unprocessed samples of saliva and nasal swabs

Key elements of qualitative assay

- Use of Volcano2G polymerase (amplifies both RNA and DNA templates)
- Incorporation of fluorescent reporter probe
- Readout: visual with high signal to noise ratio (blue light excitation)
- Amplifications using a standard thermocycler



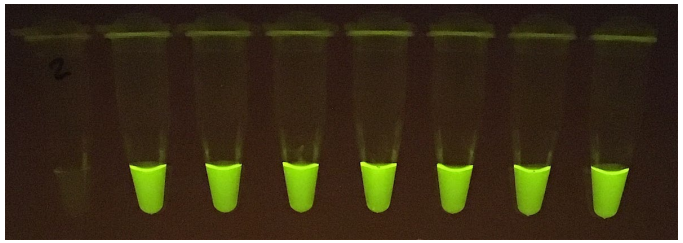
PCR amplification of SARS-CoV-2 RNA in saliva samples

- V2G polymerase efficiently amplifies target in unprocessed saliva
- Detection is highly sensitive

Subject 106

Saliva volume (µl)

NTC 1 3 5 POS



Input copies (diluted)

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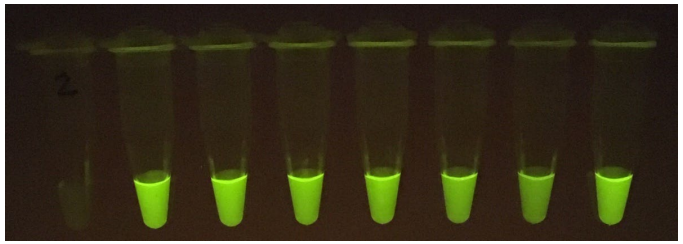

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Saliva volume (µl)

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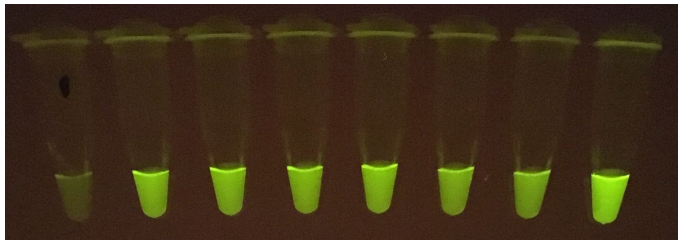

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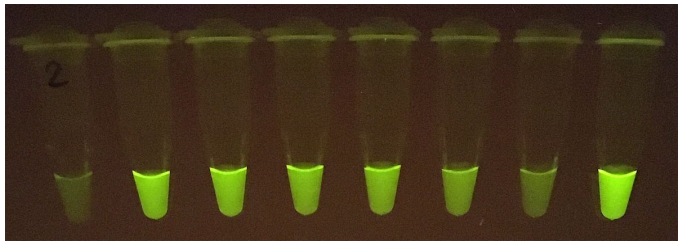
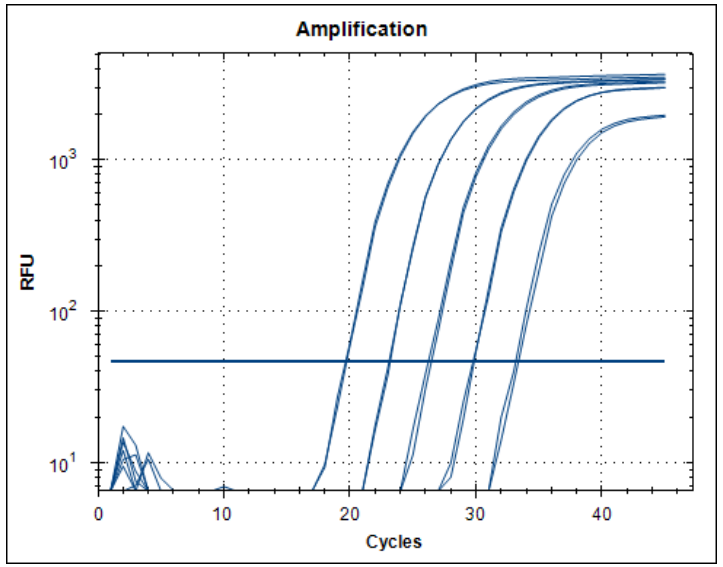
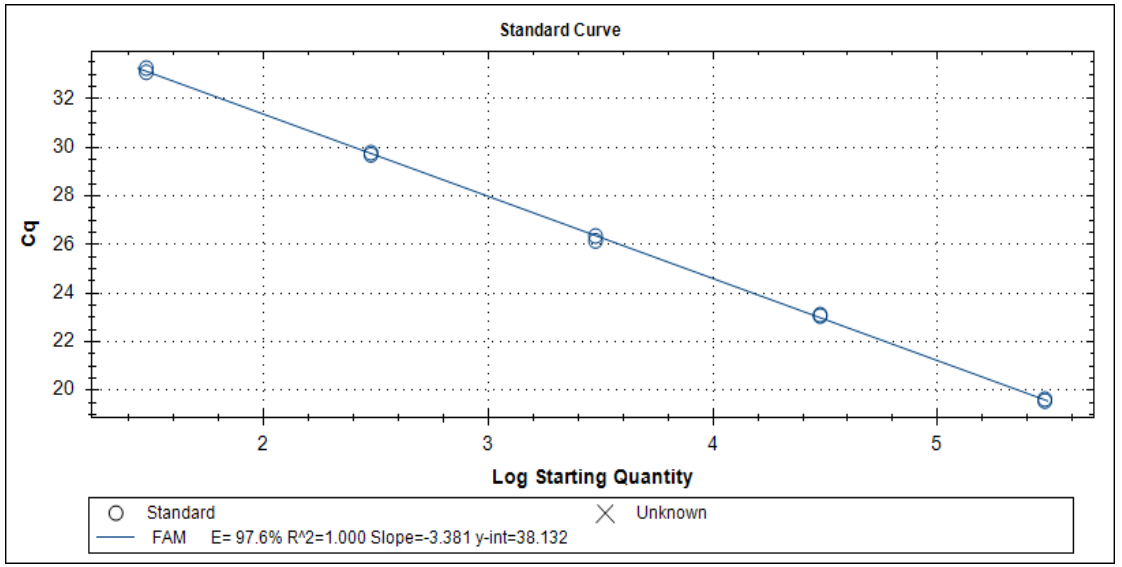


Detection of SARS-CoV-2 in wastewater samples

- Recruited by George Grills
- How does V2G PCR compare to more mainstream RT-qPCR approaches
- Compare qPCR data to that determined by group at Sylvester Cancer Center (Sion Williams)
- Basic assay was modified so it could be used to quantiatively measure CoV-2 RNA in wastewater extracts





- Data has been generated using weekly samples from the UM campus, UM hospital, CDWW treatment facility
- V2G-qPCR data correlated very well with data generated by the Williams group at Sylvester
- Published or presented by others

Controls for detection of SARS-CoV-2 in wastewater

- V2G-qPCR assays have been developed for additional targets used as controls
- All assays have similar efficiencies and detection sensitivities

Target	Function
OC43 betacoronavirus	**Processing/ extraction control**
HIV-1 RNA spike	**Indicator of PCR inhibition**
Human beta-2-microglobulin	**Normalization of SARS-CoV-2 signal**
Pepper Mild Mottle Virus	**Normalization of SARS-CoV-2 signal**

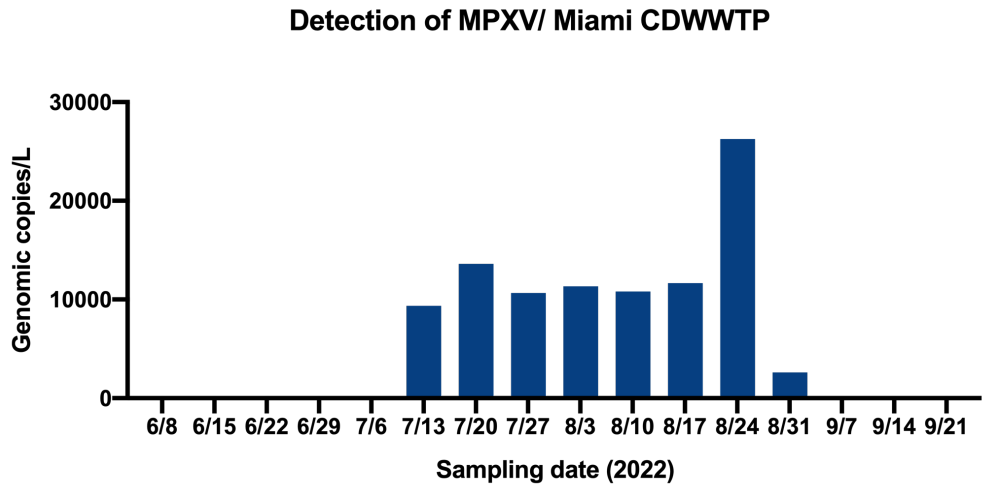
UM wastewater biorepository and other human pathogens

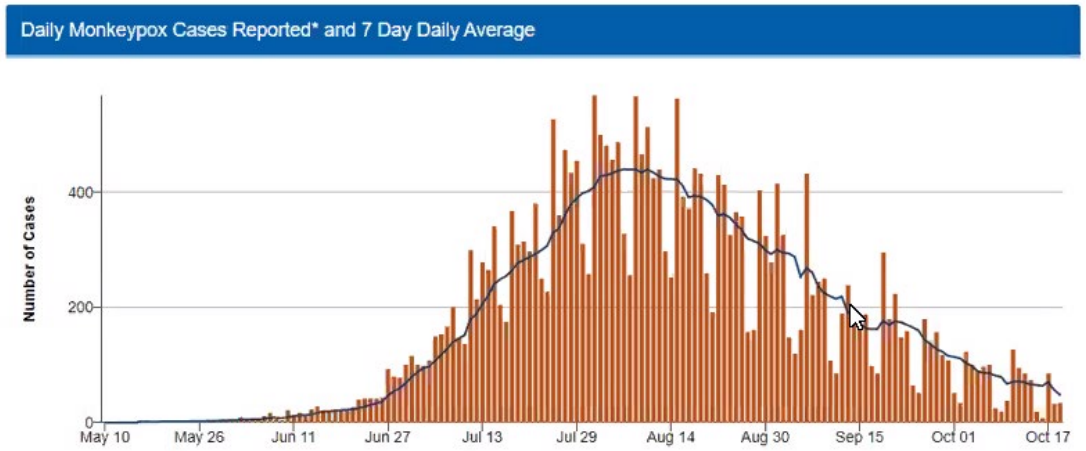
- We have a unique and important collection of samples stored
- Weekly wastewater concentrates spanning two years
- Surplus RNA extracts
- DNA extracts from 5/18/22 to 9/21/22
- Additional unprocessed concentrates for DNA extractions

- Great resource for analysis for other pathogens of interest
- Monkeypox virus and Candida auris

Detection of Monkeypox virus (MPXV) in wastewater

- In the spring of 2022, cases of MPXV infection outside of endemic regions (Africa) were reported
- Recent data suggests MPXV shedding occurs and virus could be discarded into wastewater
- Developed and utilized a qPCR assay to detect MPXV DNA in wastewater
- Miami-Dade wastewater surveillance data correlates well with the CDC national case statistics

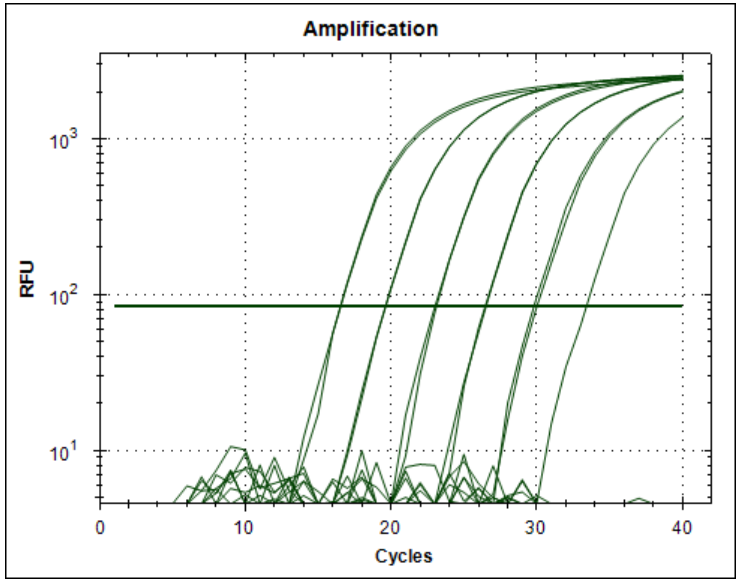


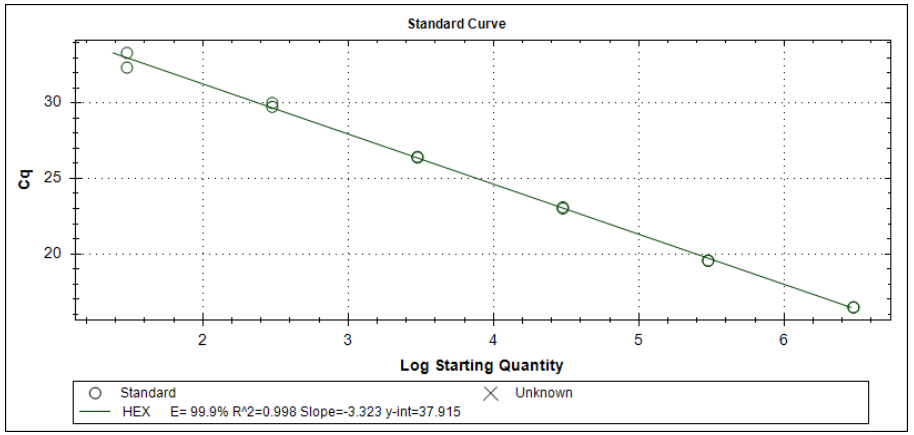


Detection of Candida auris in wastewater

- Species of fungus that grows as yeast/ first identified in 2009
- Causes fungal infections in humans affecting bloodstream, CNS and internal organs
- Clinically concerning due to multidrug resistance
- Most prevalent in hospitals/nursing homes and immunocompromised individuals

- qPCR assay targets the ribosomal RNA gene and internal transcribed spacer



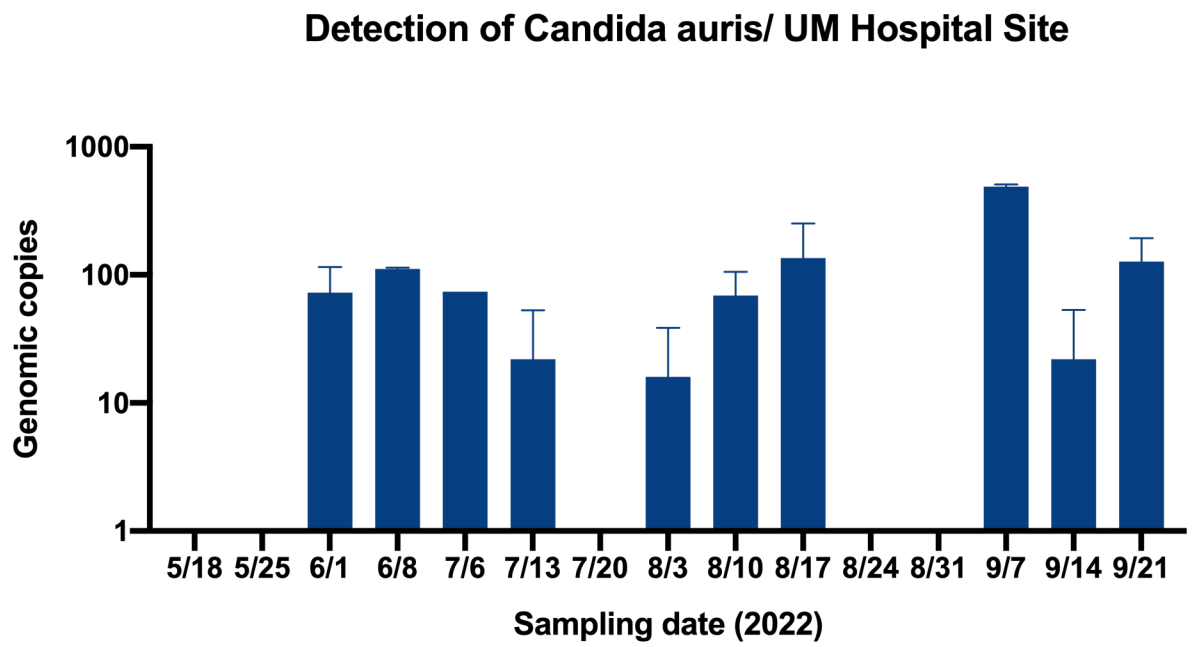


Slope -3.323 E=99.9% R^2=0.998

Detection of Candida auris in wastewater

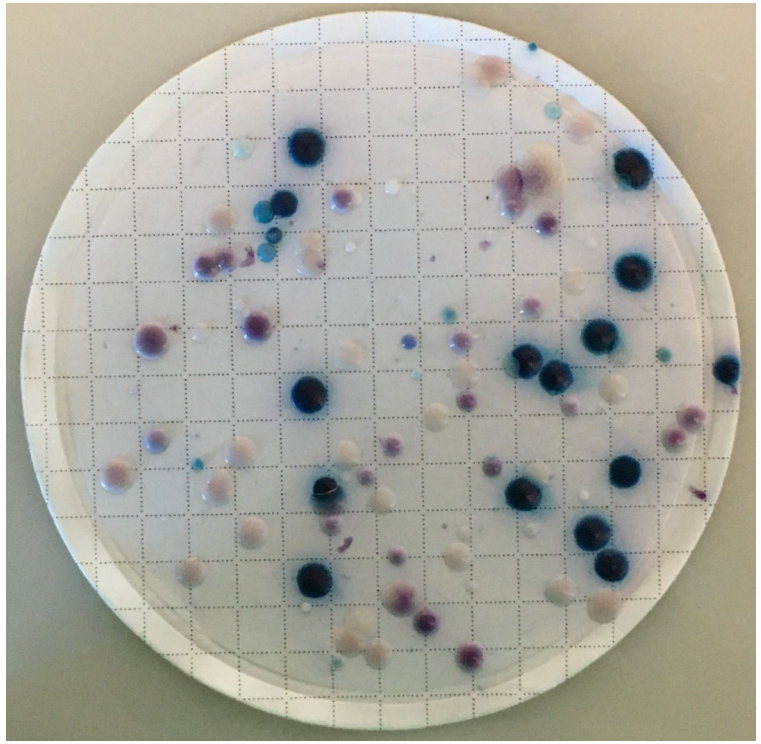
- Archival extracts were tested for the presence of Candida auris DNA
- Detectable in 10 of 15 weekly samples collected at UM hospital
- Also detected at the CDWWTP

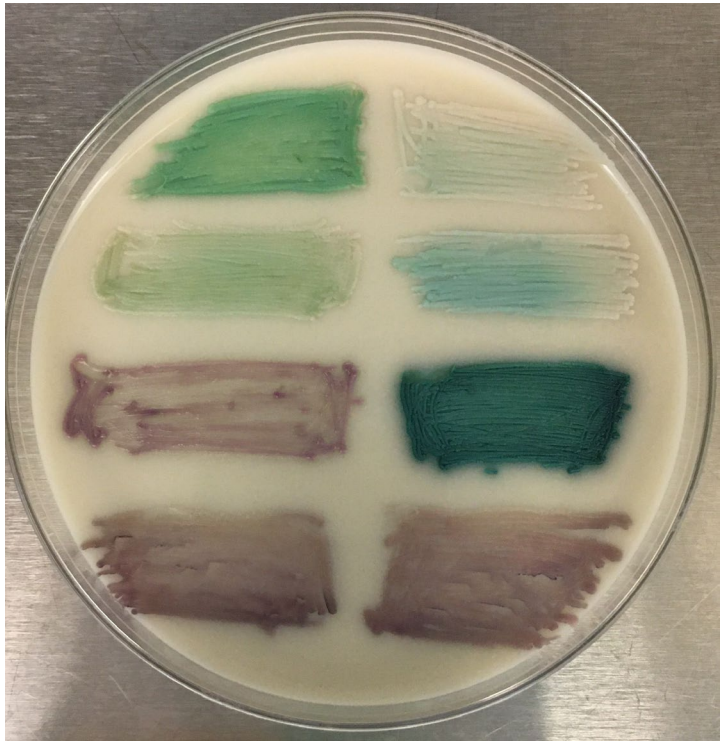
- Is the yeast in wastewater viable?



Detection of Candida auris in wastewater

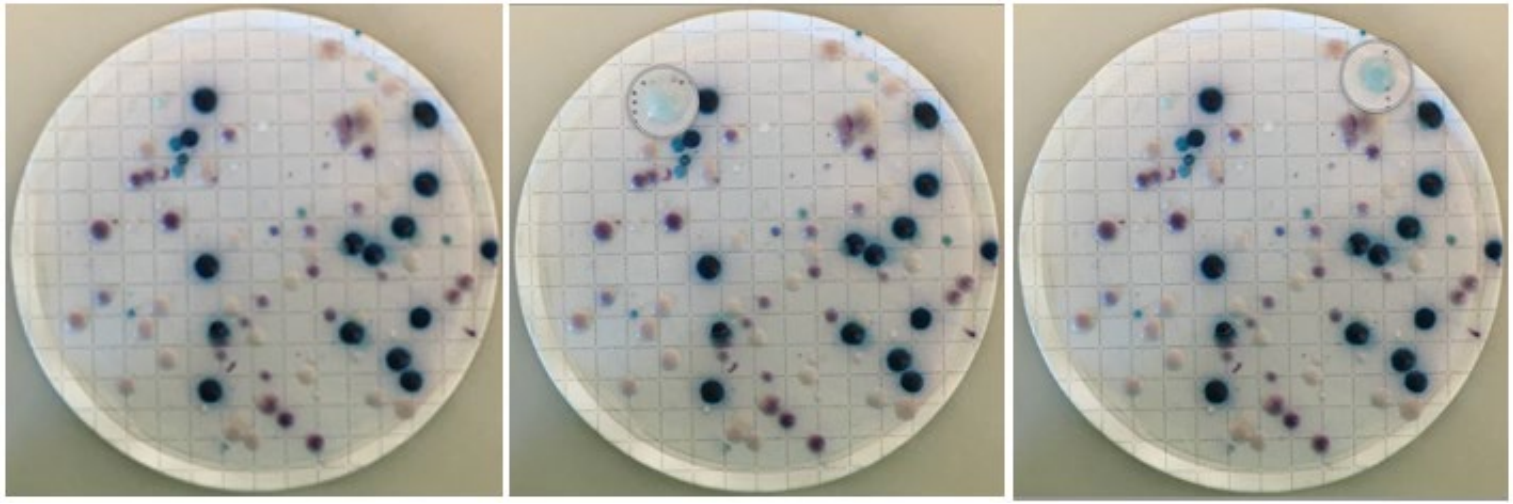
- Wastewater from UM hospital concentrated onto filter
- Placed onto chromogenic media plate
- Colonies re-streaked onto indicator plates
- DNA extracted from each/ qPCR for C. auris/ positives ✓

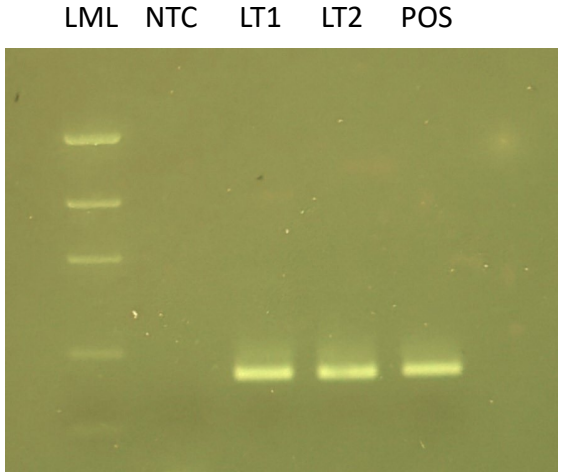




Detection of Candida auris in wastewater

- Faintly teal colonies were positive
- All others were qPCR negative
- Reagents are specific for C. auris
- C.auris is being introduced into wastewater and is viable





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Summary

- V2G-qPCR simplifies detection of CoV-2 RNA in wastewater by reducing assay time and cost
- CoV-2 RNA levels measured by V2G-qPCR correlate well with more mainstream RT-qPCR methods to quantify CoV-2 RNA
- Analyses using V2G can easily be expanded to detect an array of RNA targets
- Targeted PCR amplification is an effective and sensitive method to detect pathogen nucleic aids in wastewater
- Wastewater based surveillance provides a noninvasive, cost-effective approach to monitor communities for pathogens
- Archival samples are available to test for other microbes of interest
- Introduction of pathogens into wastewater that are viable may be a concern for community health.

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