BIOMÉRIEUX

The Combined Utility of the BIOFIRE® FILMARRAY® Research Configurations for Influent Wastewater Surveillance.

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INTRODUCTION

Wastewater-based surveillance is the intentional and consistent measurement of health markers in a wastewater or wastewater-derived sample. The BIOFIRE® FILMARRAY® qPCR system uses diagnostic panels designed to detect many human pathogens from a single patient sample of various sample types. The system includes integrated sample preparation, nucleic acid extraction, nested multiplex PCR, and melt curve analysis.

METHODS

To assess the performance of the BIOFIRE® system, two research configurations of existing BIOFIRE® respiratory, pneumonia, and enteric assays were used to monitor influent wastewater. Three study sites were set up throughout 2023. These sites included the Universitat de Barcelona (UB), SUEZ, and the Utah Department of Health and Human Services (UDHHS). These assays were chosen to assess their ability to detect the presence of analytes such as SARS-CoV-2, Influenza A/B, and Respiratory Syncytial Virus (RSV) in wastewater. Other assays including those for seasonal viruses, bacteria, fungi, and protozoa were also assessed in this study. Testing was performed with concentrated and unconcentrated influent wastewater samples following each site's standard preparation methods. For UDHHS, Crossing point (Cp) analysis was performed for Influenza and RSV using the BIOFIRE® FIREWORKS™ software (figure 4) to generate trend results which were then compared to clinical incidence rates in Utah (figure 5).
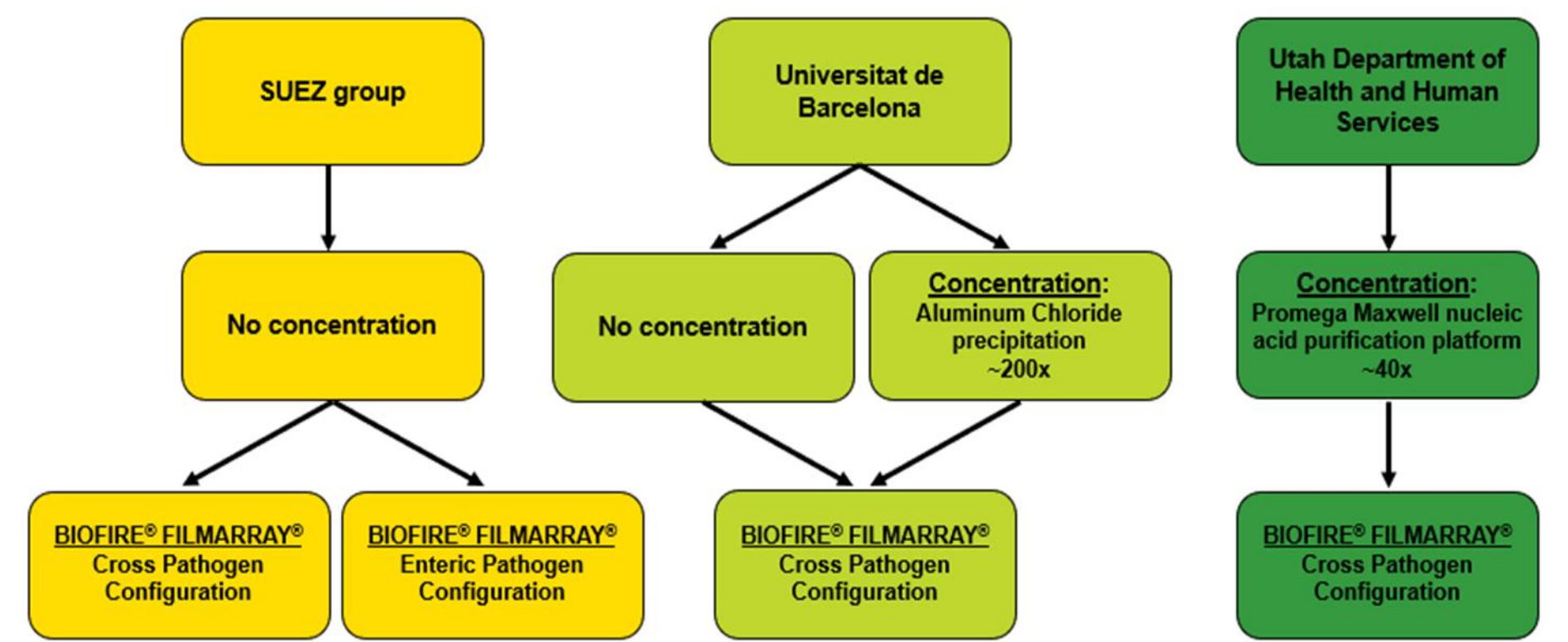
The BIOFIRE® FILMARRAY® System Wastewater Research Configurations Target Assays

Research Configuration 1 - Cross Pathogen / AMR Target List		Research Configuration 2 - Enteric Pathogen Target List	
Bacteria	**Viral**	**Bacteria**	**Viral**
Acinetobacter Calcoaticus-baumannii complex	Adenovirus	Campylobacter	Adenovirus F40/41
Bordetella parapertussis	Seasonal Coronavirus (Non-SARS-CoV-2)	Clostridioides (Clostridium) difficile (toxin A/B)	Astrovirus
Bordetella pertussis	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Plesiomonas shigelloides	Norovirus GI/GII
Chlamydia pneumoniae	Human Metapneumovirus (hMPV)	Salmonella	Rotavirus A
Enterobacter cloacae complex	Human Rhinovirus/Enterovirus (HRV/EV)	Yersinia enterocolitica	Sapovirus (I, II, IV, and V)
Escherichia coli	Influenza A virus (FluA)	Vibrio spp.	
Haemophilus influenzae	Influenza B virus (FluB)	Vibrio cholerae	
Klebsiella aerogenes	Middle Eastern Respiratory Virus (MERS)		
Klebsiella oxytoca	Parainfluenza virus (PIV)	**DIARRHEAGENIC ESCHERICHIA COLI/SHIGELLA:**	
Klebsiella pneumoniae group	Respiratory syncytial virus (RSV)	Enteroaggregative E. coli (EAEC)	
Legionella pneumophila		Enteropathogenic E. coli (EPEC)	
Moraxella catarrhalis	**Antimicrobial Resistance Genes (AMR):**	Enterotoxigenic E. coli (ETEC) lt/st	
Mycoplasma pneumoniae	Carbapenemases: ESBL:	Shiga-like toxin-producing E. coli (STEC) stx1/stx2	
Pseudomonas aeruginosa	IMP CTX-M	E. coli O157	
Proteus spp.	KPC	Shigella/Enteroinvasive E. coli (EIEC)	
Serratia marcescens	NDM		
Staphylococcus aureus	OXA-48-Like	**Parasites**	
Streptococcus agalactiae	VIM	Cryptosporidium	
Streptococcus pneumoniae		Cyclospora cayetanensis	
Streptococcus pyogenes	**Methicillin resistance:**	Entamoeba histolytica	
Stenotrophomonas maltophilia	mecA/C and MREJ (MRSA)	Giardia lamblia	

SUEZ RESULTS

SUEZ

SUEZ - Cross Pathogen Configuration			
Type	Assay Name	Positive Detections (n=25)	Percent Positive Detection
Viral	Adenovirus	25	100%
	Seasonal CoV	3	12%
	FluA	3	12%
	FluB	0	0%
	hMPV	0	0%
	HRV/EV	25	100%
	MERS	0	0%
	PIV	5	20%
	RSV	4	16%
	SARSCoV2	25	100%

SUEZ - Cross Pathogen Configuration			
Type	Assay Name	Positive Detections (n=25)	Percent Positive Detection
Bacterial	Acinetobacter	25	100%
	C. pneumoniae	1	4%
	E. cloacae	24	96%
	E. coli	25	100%
	H. influenzae	0	0%
	K. aerogenes	21	84%
	K. oxytoca	25	100%
	K. pneumoniae	25	100%
	L. pneumophila	4	16%
	M. catarrhalis	0	0%
	M. pneumoniae	0	0%
	P. aeruginosa	23	92%
	P. roteus spp.	24	96%
	S. agalactiae	22	88%
	S. aureus	22	88%
	S. maltophilia	24	96%
	S. marcescens	22	88%
	S. pneumoniae	0	0%
	S.pyogenes	0	0%

SUEZ - Cross Pathogen Configuration			
Type	Assay Name	Positive Detections (n=25)	Percent Positive Detection
AMR	CTX-M	25	100%
	IMP	25	100%
	KPC	21	84%
	MRSA+	24	96%
	NDM	25	100%
	OXA-48-like	25	100%
	VIM	25	100%

Table 1. BIOFIRE® FILMARRAY® Cross Pathogen Configuration SUEZ positivity results for non-concentrated 24-hour composite influent wastewater samples collected August – November 2023 (n=25).

SUEZ - Enteric Pathogen Configuration			
Type	Assay Name	Positive Detections (n=28)	Percent Positive Detection
Viral	Adenovirus Type F	28	100%
	Astrovirus	27	96%
	Norovirus	27	96%
	Rotavirus A	26	93%
	Sapovirus	28	100%

SUEZ - Enteric Pathogen Configuration			
Type	Assay Name	Positive Detections (n=28)	Percent Positive Detection
Bacterial	Aeromonas spp.	27	96%
	Campylobacter spp.	22	79%
	C. difficile	6	21%
	P. shigelloides	9	32%
	Salmonella	8	29%
	Shigella spp.	14	50%
	V. cholerae	9	32%
	Vibrio spp.	13	46%
	Y. enterocolitica	21	75%

SUEZ - Enteric Pathogen Configuration			
Type	Assay Name	Positive Detections (n=28)	Percent Positive Detection
Parasites	C. cayetanensis	0	0%
	Cryptosporidium spp.	4	14%
	E. histolytica	2	7%
	G. lamblia	28	100%
DIARRHEAGENIC ESCHERICHIA COLI/SHIGELLA	Enteroaggregative (EAEC)	28	100%
	Enteropathogenic (EPEC)	28	100%
	Enterotoxigenic (ETEC)	28	100%
	Shiga-like toxin-producing (STEC)	27	96%
	STEC + O157	20	71%

Table 2. BIOFIRE® FILMARRAY® Enteric Pathogen Configuration SUEZ positivity results for non-concentrated 24-hour composite influent wastewater samples collected August – November 2023 (n=28).

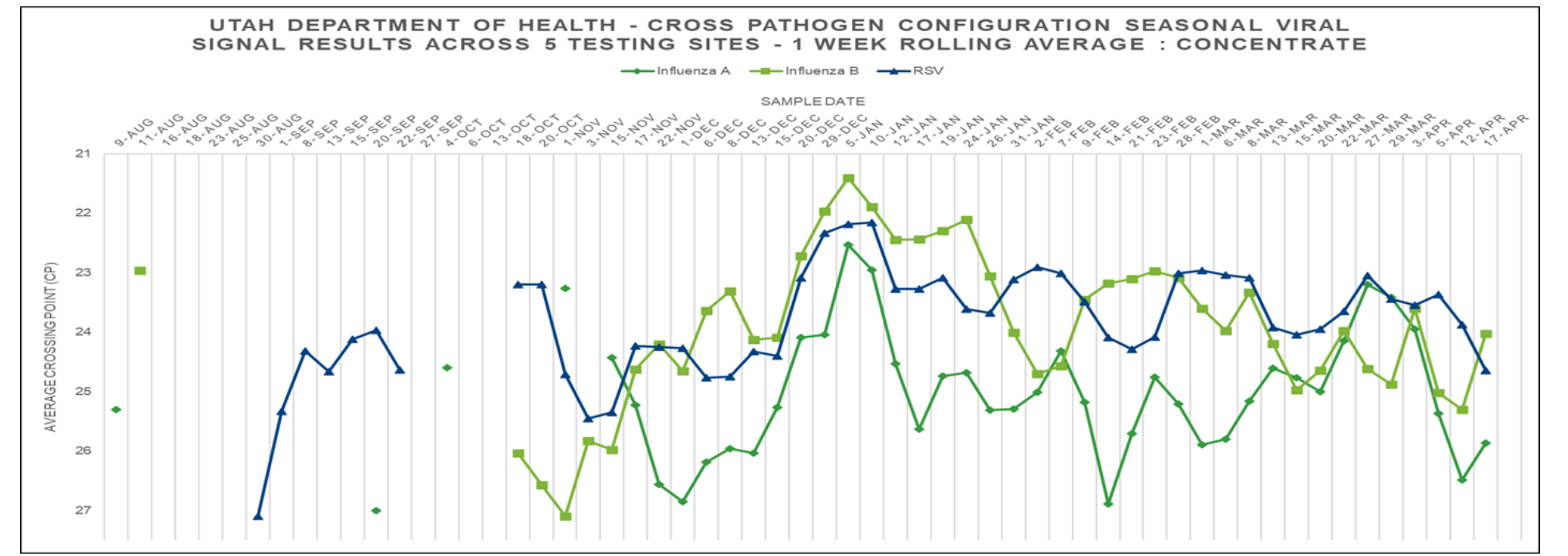## UTAH DEPARTMENT OF HEALTH AND HUMAN SERVICES

Figure 4. BIOFIRE® FILMARRAY® Cross Pathogen Configuration UDHHS seasonal viral results 1 week rolling average across 5 wastewater collection sites for concentrated 24-hour composite influent wastewater samples Salt Lake County collected August 2023 – April 2024 (n=288).
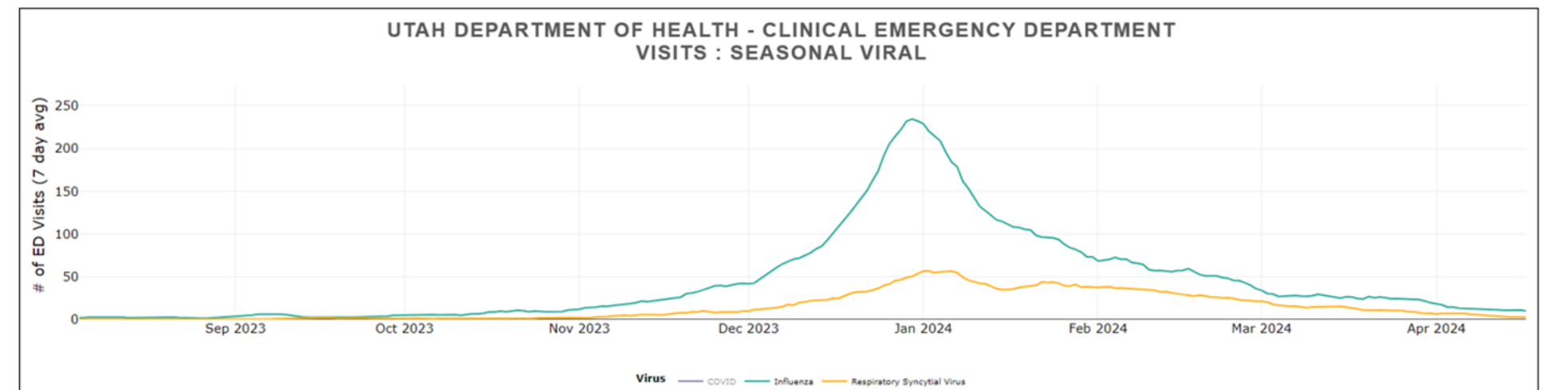

Figure 5. Statewide emergency department visits attributed to influenza and RSV reported by the Utah department of health and human services (https://coronavirus.utah.gov/case-counts/)
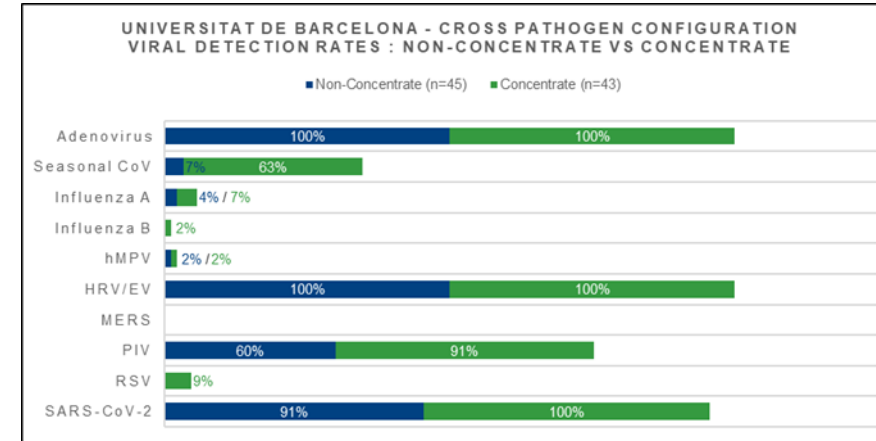
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Figure 1. BIOFIRE® FILMARRAY® Cross Pathogen Configuration UB viral detection results for non-concentrated and concentrated 24-hour composite influent wastewater samples collected April – July 2023.
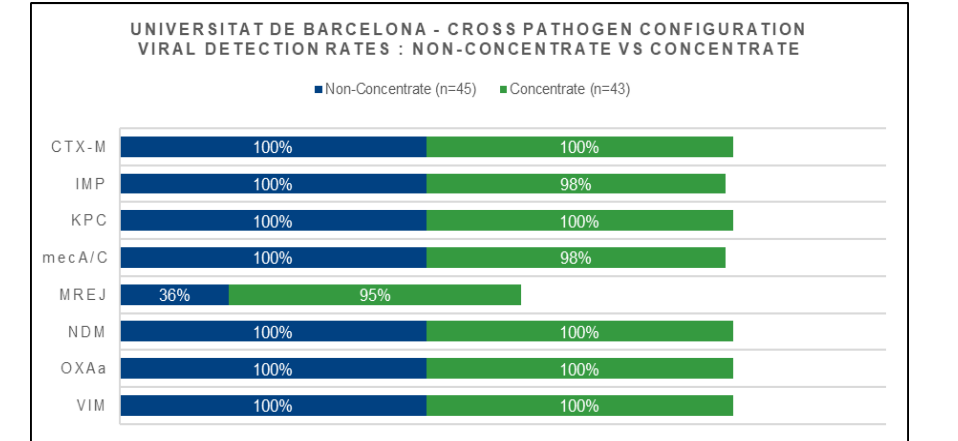

Figure 2. BIOFIRE® FILMARRAY® Cross Pathogen Configuration UB AMR detection results for non-concentrated and concentrated 24-hour composite influent wastewater samples collected April – July 2023.
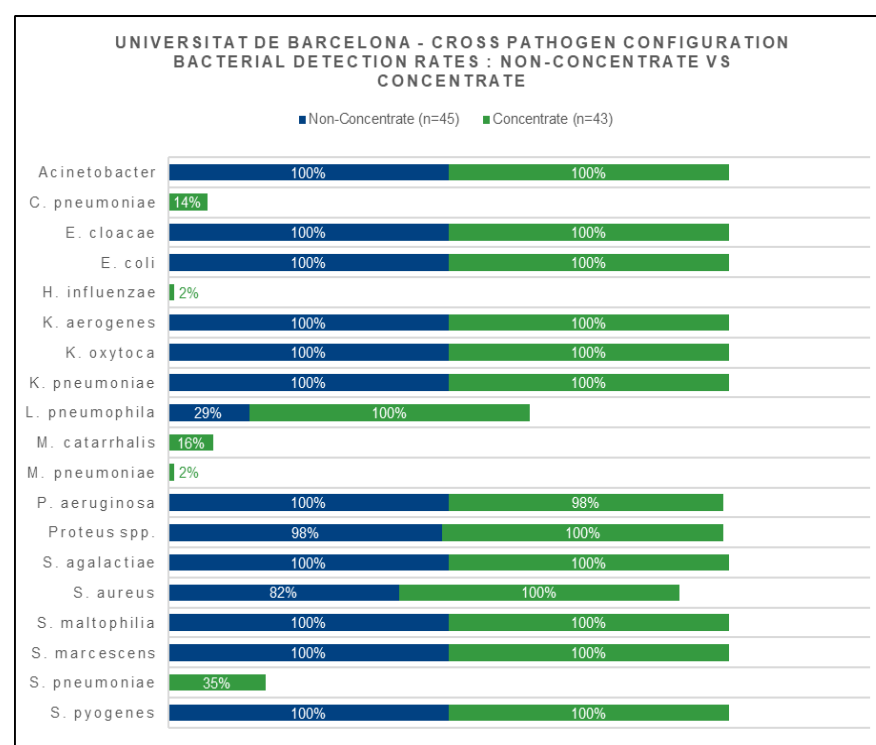

Figure 3. BIOFIRE® FILMARRAY® Cross Pathogen Configuration UB bacterial positivity results for non-concentrated and concentrated 24-hour composite influent wastewater samples collected April – July 2023.

AMR Gene Result	Applicable Bacteria
mecA/C and MREJ	Staphylococcus aureus
CTX-M IMP KPC NDM VIM	Acinetobacter calcoaceticus-baumannii Complex
	Escherichia coli
	Enterobacter cloacae complex
	Klebsiella aerogenes
	Klebsiella oxytoca
	Klebsiella pneumoniae group
	Proteus spp.
	Pseudomonas aeruginosa
	Serratia marcescens
OXA-48-like	Enterobacter cloacae complex
	Escherichia coli
	Klebsiella aerogenes
	Klebsiella oxytoca
	Klebsiella pneumoniae group
	Proteus spp.
	Serratia marcescens

Table 3. AMR gene targets and associated organisms.

Universitat de Barcelona Sample Sites	
Site	Population
Tarragona	145,832
Girona	153,162
Besós	1,829,978
Prat de Llobregat	1,181,412
Terrassa	250,023

Table 4. Population per testing site UB.

GLOBAL RESULT SUMMARY

For influent wastewater samples across all testing sites, SARS-CoV-2 was detected in approximately 90% of unconcentrated samples and in 99% of concentrated samples tested. Influenza and RSV were mainly detected throughout respiratory season. For site Utah, Increasing wastewater signal was observed for Flu and RSV from mid-October peaking on January 5th corresponding with clinical incident rates for the state. Environmental presence of these Viral analytes continued through April. Regular detections were observed for Adenovirus, HRV/EV, seasonal coronaviruses (non-SARS-CoV-2), Parainfluenza virus, Norovirus, Astrovirus, Rotavirus, Sapovirus, *Bordetella parapertussis*, *Giardia lamblia*, Diarrheagenic *E. coli/Shigella*, and *Yersinia enterocolitica*. Furthermore, non-listed bacterial enteric pathogen assays showed high positivity rates in influent wastewater samples, as expected.

CONCLUSIONS

BIOFIRE® research configurations showed robust analyte detection with influent wastewater samples. These feasibility results suggest that the BIOFIRE® assays can be used for the intended purpose of wastewater-based surveillance.

ACKNOWLEDGEMENTS