Developing internal process controls to support analytical rigor for wastewater and environmental surveillance methods

Biosafe standards to qualify wastewater testing methodologies

Introduction

The recent COVID-19 pandemic highlighted that wastewater and environmental surveillance (WES) is a powerful tool to estimate the presence and amount of infectious disease within a community. Its greatest value is when clinical data is limited or absent and so WES provides population data for health authorities to consistently track disease and assess the risk to the community over time.

WES methods currently use microbes commonly found in sewage as an internal control for performance indicators. The number of control microbes per sample can be quite different due to dilution (e.g. rainfall), population size, seasonality, and other factors. With this variability, it is unclear whether differing control test results are due to variance in the number of control microbes or also due to other factors such as confounding substances. These are present in many wastewater samples and interfere with pathogen detection and enumeration by inhibiting sample preparation, analysis or both. Without uniform standards, it is difficult to confirm that WES samples are being processed correctly. This creates a need for standardized internal process controls (IPCs) for the routine validation of the nucleic acid extraction and amplification processes, thereby ensuring confidence in WES data.

The PATH Diagnostics Program is supported by the Gates Foundation to develop new tools to assess the performance of methods and technologies used in WES. This effort is in partnership with the UK Medicines and Healthcare products Regulatory Agency (MHRA). This project aligns with other Diagnostics Program projects supporting poliovirus WES and in creating access to key materials that support diagnostic product development and implementation.

Creating internal process controls

Following consultation with global WES experts, there was a very strong consensus that two IPCs—a bacterial IPC (bIPC; DNA target) and a viral IPC (vIPC; RNA target)—would significantly strengthen WES quality control activities, regardless of the different sampling and analytical methods being used. These IPCs provide consistent indicators of process efficiency, alert users to potential failures, and support analytical validation and method comparison.

With a bIPC created using a bacterium, users can spike prequantified amounts into samples and consistently assess the recovery of the bIPC DNA. This confirms that the method used can also effectively detect DNA from dangerous bacterial pathogens, such as typhoid or cholera. Because many of the most dangerous viruses have an RNA genome, the vIPC is made of RNA and mimics a viral genome. Similarly to the bIPC, the spiking and recovery of vIPC RNA consistently confirms that each WES sample is being correctly processed.

Both IPCs contain unique genetic sequences that are targeted by quantitative PCR assays to efficiently amplify and quantify the amount of material recovered after processing. With unique sequences, the IPCs reduce risk of false data of pathogen detection created by cross contamination.

Vials of lyophilized bIPC

MHRA/ M. Majumdar.

The IPCs design includes addressing biosafety, stability, and low cost. The bIPC is a laboratory strain of *E. coli* that is noninfectious and heat inactivated to be nonviable. The vIPC is derived from a lentivirus, to produce virus-like particles that present no infectious risk as they cannot replicate. The IPCs are quantitated and then vialed and lyophilized in specific concentrations at MHRA. Their lyophilization allows stability out of the cold chain, greatly reducing shipping costs to facilitate global deployment and support WES implementation across low-resource settings.

The project team is collaborating with groups in sub-Saharan Africa, Switzerland, and the United States to integrate the IPCs into their routine WES testing activities to assess their use and performance. This will independently validate the effectiveness of the IPCs and may confirm preliminary test data from UK wastewater where the bIPC has significantly less sample-to-sample variance than the current *Bacteroides* process control.

For more information

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