Effective concentration: a future-proof approach to characterizing pathogen concentrations in wastewater

Executive Summary
- Wastewater-based epidemiology is an important tool for monitoring Covid-19 activity.
- The raw concentration of SARS-CoV-2 as measured in wastewater is affected by technical factors unrelated to the spread of the virus.
- Currently, Biobot uses normalization to adjust for dilution and population size. As wastewater-based epidemiology advances, Biobot will adjust for more technical factors beyond dilution and population size.
- Biobot is introducing the concept of ***effective concentration*** as a transparent and future-proof approach for this gradual increase in the factors accounted for.
- Presenting the effective concentration, rather than an ever-shifting methods-specific nomenclature, will allow us to continuously improve our methods without affecting customer data interpretation.

The *raw* concentration of SARS-CoV-2 in wastewater is affected by factors unrelated to the spread of the virus

In Covid-19 wastewater-based epidemiology, the key measurement is the concentration of SARS-CoV-2 in wastewater. At Biobot, this concentration is measured using quantitative polymerase chain reaction (qPCR) and reported in units of genome copies per liter of wastewater. For clarity, we refer to this value as the *raw concentration* of a wastewater sample.

The raw concentration of a sample is the starting point for many applications of wastewater-based epidemiology. However, the raw concentration is subject to factors that are not relevant to Covid-19 disease activity, such as:
- Dilution of wastewater due to precipitation events or other inflow & infiltration
- Changes in the size of the population contributing to the sampled wastewater
- Changes in wastewater flow rates due to changes in water usage patterns
- Temperature- and time-dependent degradation of the virus in wastewater
- Recovery efficiency (i.e., proportion of virus in the sample that the laboratory sample preparation process is able to capture for quantification)
- PCR inhibition (i.e., the possibility that chemicals in the wastewater sample affect the laboratory method's ability to measure the viral concentration)
- Other differences in laboratory methodologies used to measure the virus concentration

The *normalized* concentration that Biobot currently reports accounts for some, but not all, of these nuisance factors

To account for dilution, population size, and wastewater flow, a common practice in Covid-19 wastewater epidemiology is to *normalize* the SARS-CoV-2 concentration. There are two methods for normalization:

1. Use the raw concentration (*RC*), flow rate (*FR)*, and population size (*P)* to compute the *normalized concentration* $NC = \frac{RC \times FR}{P}$.

2. Use the raw SARS-CoV-2 concentration (*RC)* and the concentration of a fecal strength indicator (*FSI)* virus to compute the normalized concentration $NC = \frac{RC}{FSI}$.

Biobot uses the second normalization method, with pepper mild mottle virus (PMMoV) as the fecal strength indicator. Because the resulting value from this normalization is a ratio (i.e., a dimensionless number), Biobot multiplies by a reference concentration (C_0) so that $NC = \frac{RC}{FSI} \times C_0$ has units of concentration (i.e., genome copies per liter).

Both normalization methods are designed to adjust the raw SARS-CoV-2 concentration measured at a sampling point to better approximate the amount of virus contributed by people represented in the wastewater sample. The first method accounts for the dilution that occurs between the point of excretion and the point of sampling by multiplying by the flow rate, and it accounts for varying population size by simple division. The second method assumes that the fecal strength indicator is excreted at some fixed population-average per capita rate, so that the concentration of the indicator at the point of sampling accounts for both dilution and varying population size. If the fecal strength indicator has similar biophysical properties as the target, then the second method also accounts for recovery efficiency. If the laboratory sample preparation method has similar recovery efficiencies for the two viruses, SARS-CoV-2 and PMMoV, then taking the ratio of the resulting measurements accounts for recovery efficiency.

Introducing the *effective* concentration

Ideally, as the field of wastewater-based epidemiology advances, more of the factors affecting raw virus concentrations in wastewater can be accounted for. Normalization was an important step, accounting for some key factors affecting the public health interpretability of wastewater measurements. Future improvements will include accounting for differences in measured virus concentrations that would result from updating or changing the laboratory protocol.

However, the resulting updates to the terminology will quickly become cumbersome. "Normalization" is a single term with two well-understood meanings among wastewater experts. "Degradation-adjusted concentration" is clear and brief enough. But "concentration adjusted for laboratory protocol differences in viral quantification" is a mouthful, while "adjusted concentration" is too vague. We posit that a single term, not specific to any one factor and instead used to encompass all potential adjustments, will facilitate communicating results going forward.

Biobot is therefore introducing the term ***effective concentration*** to refer to the raw SARS-CoV-2 concentration, *adjusted for all the factors that we can currently account for*. Today, the effective concentration is the PMMoV-normalized SARS-CoV-2 concentration. As we account for more factors, the necessary adjustments will be rolled into the algorithm used to compute the effective concentration.

For example, we continue to update and improve our laboratory protocols and operations, and any update to a protocol means that the same wastewater sample will yield different raw concentrations. During a protocol change, we measure the same wastewater samples with both the previous and new protocols. Using the resulting data, we derive an adjustment factor that accounts for differences in measured concentration due to the protocol change. The effective concentration of a sample measured using the new protocol will then reflect the concentration that would have been measured using the previous protocol.

More broadly, Biobot intends for the effective concentration to be formulated such that trends in effective concentration:
1. **Do** reflect trends in the population-wide Covid-19 burden
2. **Do not** reflect technical artifacts like differences in lab protocols

The effective concentration will not be a "black box." Biobot will continue to report raw concentrations to our customers and will note any changes to the methodology used to compute the effective concentration in public release notes. Our goal is to allow technical experts to scrutinize the raw data while providing a seamless data stream to users focused on the public health implications of the data.

An analogy: "feels like" temperatures
We chose the term "effective concentration" because it was not specific to any methodology. This approach was inspired by the *"feels like" temperatures* reported by consumer weather services.

The *raw* temperature, measured in degrees Fahrenheit or Celsius, is the most important single number for understanding how it will feel when you go outside. But other factors, like wind speed and humidity, are important too. The *wind chill temperature* is computed using the raw temperature and wind speed, while the *heat index* is computed using the raw temperature and humidity. The combined indicator, that accounts for both wind speed and humidity, is called *"feels like" temperature*.

Just as "feels like" avoids clunky phrasing like "wind- and humidity-adjusted temperature", we hope that effective concentration can avoid imprecise and confusing nomenclature while simultaneously allowing for continual improvement in our laboratory methods.