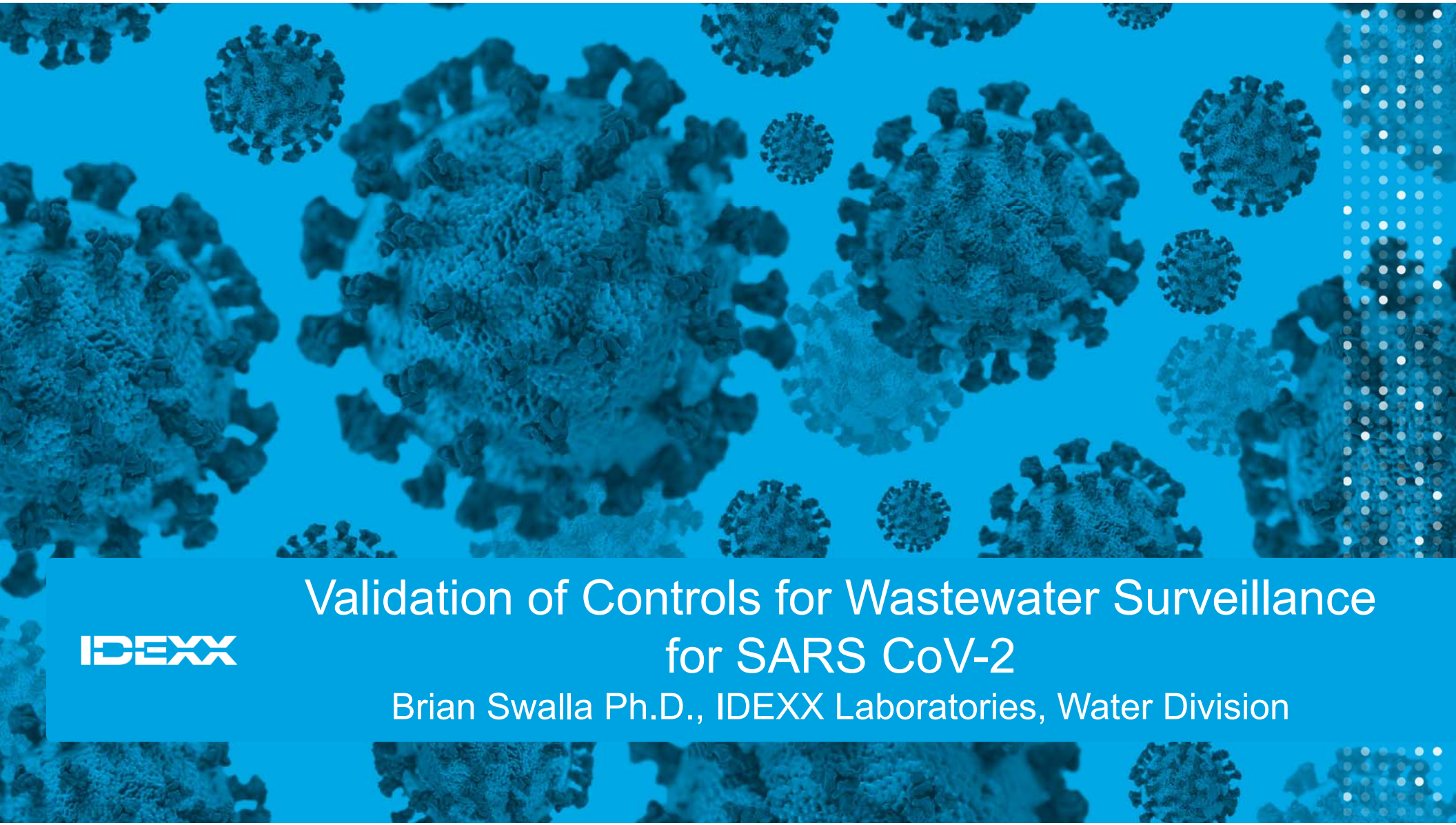


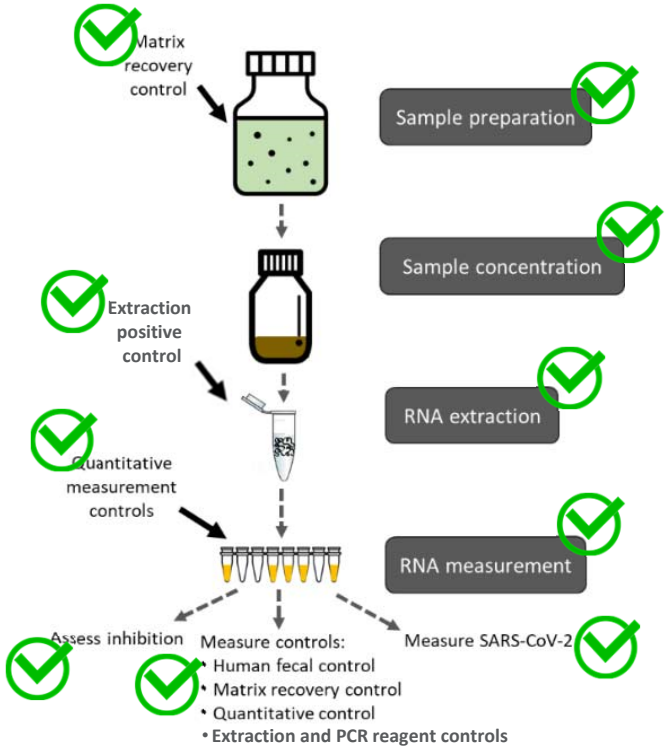
Validation of Controls for Wastewater Surveillance for SARS CoV-2

Brian Swalla Ph.D., IDEXX Laboratories, Water Division

IDEXX has invested heavily in developing materials, protocols, and data to support wastewater surveillance

IDEXX Reagents

IDEXX Protocols



IDEXX Validation Data

- 100+ samples
- 15+ different wastewater treatment facilities
  - Geographically diverse
  - Different processes
- Testing over several months
- Multiple analysts
- Different material & reagent lots
- Varied lab equipment

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IDEXX has experience implementing wastewater surveillance around the world with various types of laboratories

Countries with laboratories that have implemented or are evaluating IDEXX reagents and protocols

Customer Types

- Public Health Labs
- National Public Health Organizations
- Independent Laboratories
- Major contract laboratories
- Academic Research Labs
- Universities (on campus testing)
- Wastewater Utilities

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Validation data allows labs with robust quality procedures to produce results that can be used for public health decisions

Validation

- Method developer characterizes performance under different conditions
  - Laboratory
  - Analyst
  - Type of sample
  - Geography
  - Etc…

Lab Verification

- Laboratory testing confirms method performance compared to validation data

Quality SOP

- Ongoing quality control procedures to ensure consistent performance

Because of the urgency of the pandemic, validation has often been limited in scope, which can lead to unintended consequences

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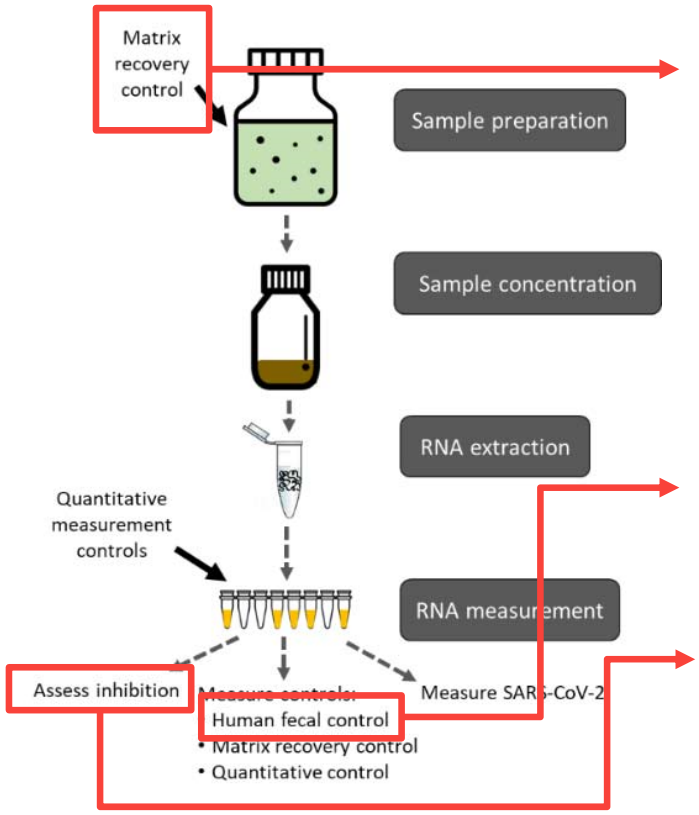
IDEXX's experience with wastewater surveillance has demonstrated the importance of validation

Matrix Recovery

Fecal Normalization

Internal Control



Matrix Recovery Control

- Importance of material choice

Human Fecal Control

- Importance of probe design

Internal Control

- Ability to assess inhibition

Validation data should drive these decisions

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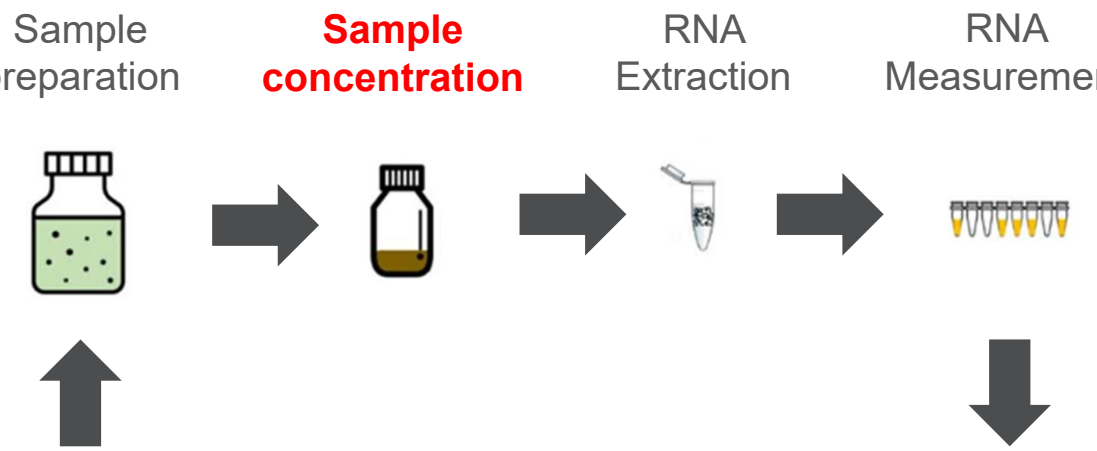
A matrix recovery control is critical for assessing yield loss during concentration

Matrix Recovery
Fecal Normalization
Internal Control



Spike known amount of exogenous material

Measure remaining material to calculate yield

Sources of variation in concentration

- Relatedness of spike to SARS-CoV-2
- Inherent variability of wastewater matrix
- Variability of wastewater inputs (non-Human sources)
- Run-to-run variation

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IDEXX testing has demonstrated that certain materials perform differently in wastewater

Matrix Recovery

Fecal Normalization

Internal Control

Collection Date	Site	Sample	BRSV Recovery %		
			Replicate		Average
			A	B	
Day 1	A	260	69.3%	49.1%	59.2%
Day 3		259	34.5%	38.7%	36.6%
Day 1	B	275	65.7%	83.5%	74.6%
Day 2	C	266	15.9%	15.1%	15.5%
Day 4		262	22.7%	20.2%	21.5%
Day 6		280	15.4%	14.6%	15.0%
Day 2	D	274	33.6%	36.6%	35.1%
Day 3		247	12.6%	13.4%	13.0%
Day 5		283	30.9%	28.7%	29.8%
Day 4	E	264	32.2%	31.4%	31.8%
Day 5	F	268	17.2%	15.1%	16.2%
Day 6	G	281	25.5%	26.5%	26.0%
			Average Recovery:		31.2%

Higher variation between sites

Recovery of Bovine Respiratory Syncytial Virus (BRSV) from wastewater using PEG-based concentration

- IDEXX data show BRSV can be used reliably as a matrix recovery control
- IDEXX also tested Bovine Coronavirus (BCoV)
- BCoV showed lower stability in wastewater and more variation in results
- However, BCoV is still widely used

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A human fecal control can account for differences in relative human waste input over time

Matrix Recovery
Fecal Normalization
Internal Control

Without human fecal control

Human Inputs

Human Inputs
Rain Event
Industrial/Other Inputs

Higher concentration

Lower Concentration

Poor public health decisions

With human fecal control

Human Inputs

Human Inputs
Rain Event
Industrial/Other Inputs

Human Fecal Normalization
via PMMoV or crAssphage

Normalized
concentration

Normalized
concentration

Appropriate public health decisions

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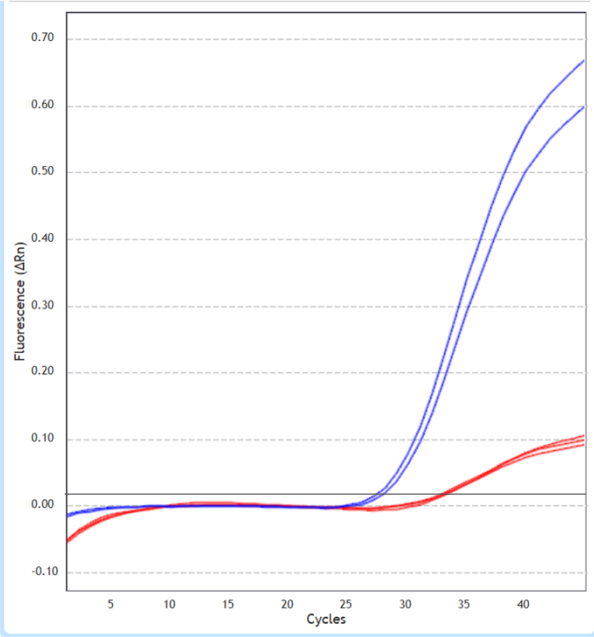
IDEXX testing demonstrated the importance of the original probe design

Matrix Recovery

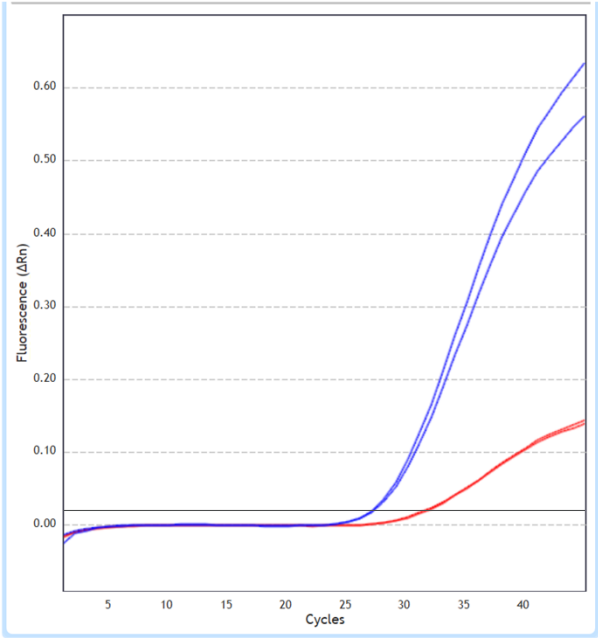
Fecal Normalization

Internal Control

sample 1



sample 2



MGB = blue no MGB = red

- The original PMMoV probe design included a “minor groove binder,” or MGB
- Some publications reported results for wastewater not using MGB
- IDEXX testing demonstrated that MGB is important for probe performance
- Methods need to be examined critically to evaluate risks

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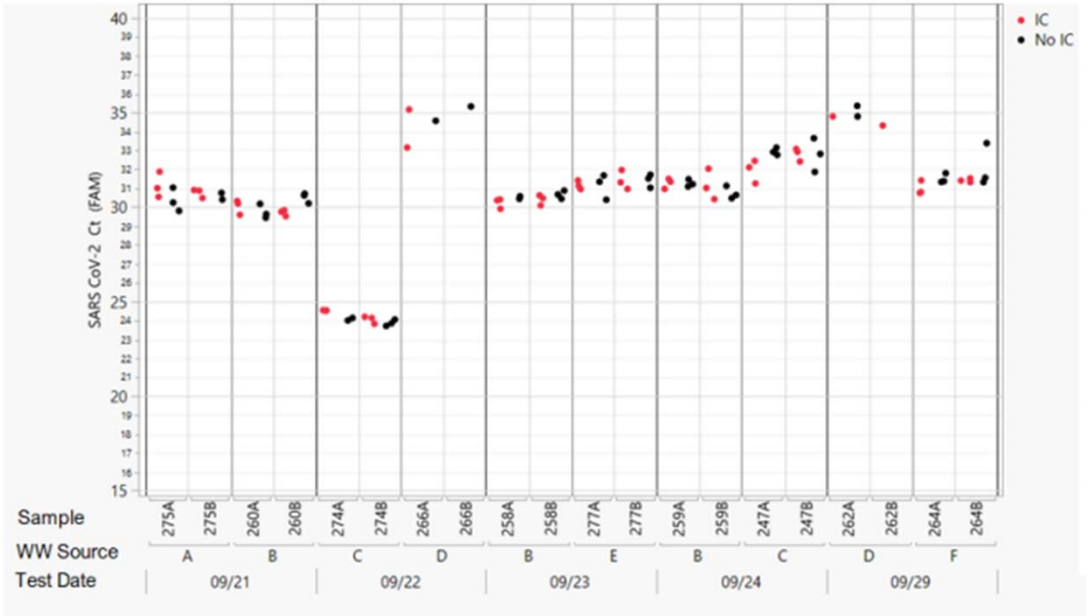
Internal controls must be validated to show no impact on quantification

Matrix Recovery

Fecal Normalization

Internal Control

- An internal control (IC) can indicate validity for each sample result, based on:
  - Inhibition potential
  - Successful purification and amplification
- The IC reaction **must** be multiplexed with the SARS-CoV-2 reaction
- There is a potential the IC reaction could affect quantification
- Validation is the **only** way to assess this risk



No impact of Multiplex Internal Control Reaction on SARS-CoV-2 Detection or Quantification

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IDEXX Laboratories, Inc.

- Headquarters in Westbrook, Maine
- 700,000+ sq. ft. manufacturing and offices
- USDA licensed; ISO 9001, 14001, 17025 certified
- Offices in 17 countries, serving customers in 120 countries
- A S&P 500 and Nasdaq 100 company

- Worldwide market leader
- Specialist in water microbiology
- Accepted or approved in 50+ countries; over 100 different approvals/acceptances by more than 20 entities globally
- **Helping protect water quality for an estimated 2.5 billion people every day**

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IDEXX's experience in validated wastewater testing enabled rapid validation of wastewater surveillance methods

>20 years of wastewater expertise

- More than 1,500 testing systems used in wastewater facilities around the globe
- Methods approved by or included in ~20 regulatory bodies and standards

>10 years of real-time PCR expertise

- Creator of the RealPCR tests, with ~20 kits commercialized globally
- The OPTI SARS-CoV-2 RT-PCR Test Kit has received FDA emergency use authorization
- >1 million PCR tests performed each year

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Questions

Brian-Swalla@idexx.com