New NSSP Method for MSC in Effluent Samples

Working Range with Pre-disinfection and Final Effluent Samples

- A Single Pre-disinfection Effluent Sample has a Working Range from 5 to 12,000 PFU/100ml.

- Triplicate Final Effluent Samples can be combined to lower the working range to 2 to 12,000 PFU/100ml.

- LOD and LOQ of the method are 2 and 20 PFU/100ml, respectively.

- This working range has proven well-suited for the assessment and data analysis techniques.

The New ISSC Method for Enumerating MSC in Wastewater Effluent Samples

- Efficient in the Field
- Simple and inexpensive in the lab
- High Laboratory Throughput
- Direct, Non-ambiguous results

The Field Work

- An **organizational meeting** with plant operators, shellfish program manager, and field staff to get everyone on the same page.
- Sample collections occur three days a week (M,W,F) between 9-11:00 AM (Peak Flow Time).
- In the case of a significant weather or plant abnormality events further samples will be collected.

Field Equipment and Kit

Labeled 250 mL Nalgene Sample Bottles

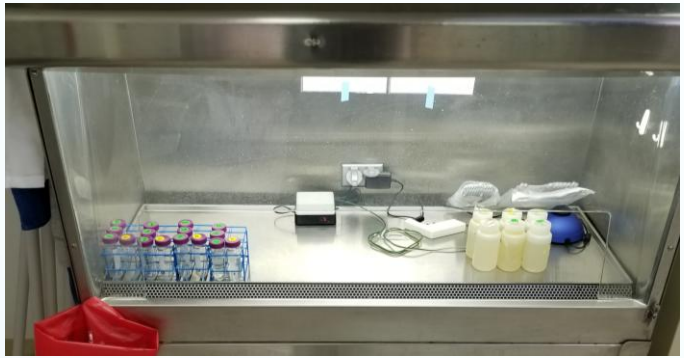
The Graduated Dipper

Complete Sample Collection Kit

Sample Collection Considerations

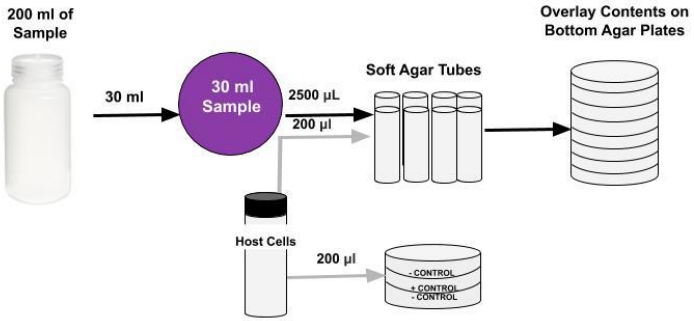
- Final effluents samples are taken prior to pre-disinfection samples to limit potential cross contamination.
- The dipper is rinsed with fresh water and sanitized after sampling each plant.
- Post chlorination samples are vigorously shaken for 30 seconds to ensures proper mixing with the 10% sodium thiosulfate solution.
- Samples are kept inside the sampling kit to maintain temperature control.

Preparation of Wastewater Samples for Analysis



- Samples are vigorously shaken (30 seconds) before being aseptically transferred into 50 ml conical tubes.
- 30 ml of each sample is transferred into its associated conical tube and vortexed for 10 seconds.
- Final effluent samples are run in triplicate. Pre-Treatment are run in single.
- Turbidity measurements are taken for all effluent samples.

Analytical Technique for WWTP Samples

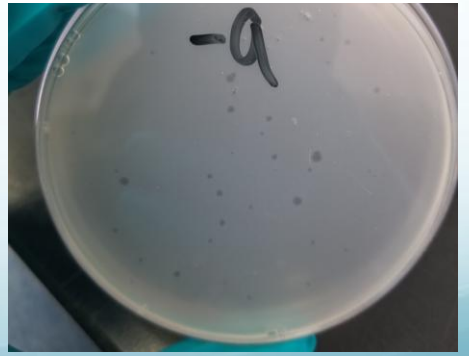


Zones of Clearing

- Plates are inverted and incubated for 16-20 hours at 35-37^{o}C.
- Scores are obtained by counting circular zones of clearing (1-10 mm in diameter) in the lawn of host bacteria.
- These are known as plaques and can be counted using a digital counter pen.

Inverted Plates in Incubator

Positive control plate displaying MSC plaques



Time Resource Management

- This method is **inexpensive** with glassware, plastic ware, agars, and reagents only costing approximately $25 per sample.
- Less than 2 hours of **field work** required to sample 3 separate WTPs within 20 minutes of each another.
- From initiation of host culture to counting plates, an estimated two and half hours of hands on **lab work** is required to test samples from 4 WTTPs.
- Four hours of **media preparation** takes place on a weekly basis.
- In total **less than 20 man hours** required for a single individual to sample and run 4-6 WTP simultaneously

So easy a caveman can do it!