

## DEMONSTRATION

by Greg Lovelace and Brian Robinson



**Goals:** Describe and demonstrate the application of:

- US EPA Method 1601 (enrichment-spot plating), US EPA Method 1602 (single agar layer plaque assay) to the detection of coliphages in shellfishing waters and shellfish tissue extracts
- current nucleic acid methods for grouping of F+ coliphages (coliphage "genotyping").

### Methods Demonstrated Through Simulation

*Method 1601* in a most probable number (MPN) format

- (1) preparation of sample medium and host bacteria
- (2) spot plating of enriched samples onto medium containing host bacteria
- (3) recognition and counting of positive spots, lysis zones on plates
- (4) computation of MPN coliphage concentrations

*Method 1602*, the single agar layer (SAL) plaque assay method

- (1) preparation of molten agar medium
- (2) inoculation of samples with host bacteria and molten agar
- (3) pouring SAL plates
- (4) observation and counting of plaques
- (5) computation of coliphage concentration.

*Confirmation Methods 1601 and 1602,*

- (1) "picking" material from positive lysis zones and plaques
- (2) resuspending it in broth medium
- (3) optional re-enrichment by overnight culture with host bacteria
- (4) inoculation of resuspended or re-enriched picked material onto spot plates to observe for appearance of lysis zones as evidence of coliphage positivity.

*Nucleic acid genotyping methods*

- (1) extraction of nucleic acids from coliphage samples
- (2) PCR or RT-PCR to amplify the concentrations of coliphage nucleic acids
- (3) application of extracted or amplified nucleic acids to filters
- (4) reaction of target nucleic acids on membranes with nucleic acid probes
- (5) detection of positive nucleic acid hybrids

*Positive reaction products from nucleic acid genotyping methods*

- (1) positive enrichment spot plates with lysis zones
- (2) SAL plates with plaques
- (3) positive nucleic acid hybrids on filters from coliphage genotyping analysis

**FOR METHODOLOGIES PLEASE SEE**  
**"Methods to Detect and Genotype Coliphages in Water and Shellfish"**  
at [www.unc.edu/sobseylab/](http://www.unc.edu/sobseylab/) under the Documents page