

## Quantitative High-Throughput Testing of Recreational Water Samples for Enterococci Using Magnetic Particle Target Capture and Transcription Mediated Amplification

Ian Weeks PhD, FRSC  
Director of Technology

## Environmental Microbiology

Current routine methods principally based on culture

• Selective agar/indicator



• Defined substrate



Results not available for at least 24 hours

## Enterococcus as an indicator of human fecal pollution in recreational water

- Southern California beaches
- State limit 104 CFU/100 ml water
- Monitoring important for human health and tourism considerations
- Require result as quickly as possible to determine action required

## Rapid, High Sensitivity Methods

Polymerase Chain Reaction (PCR) is the most talked about methodology

But there are other amplification methods

For example:

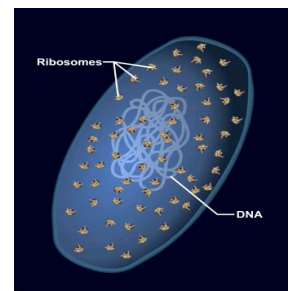
Transcription Mediated Amplification (TMA)

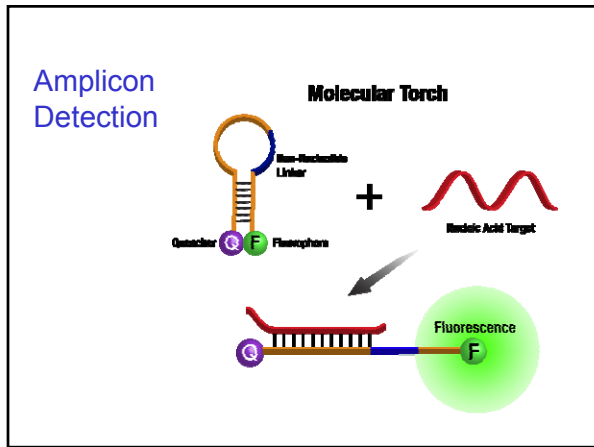
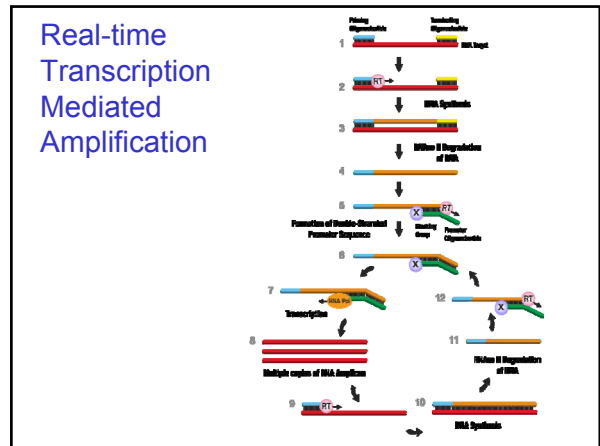
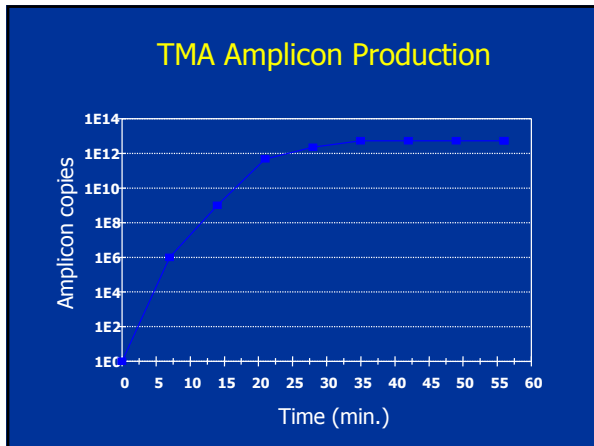
## Why use TMA?


- Successfully used in FDA-approved clinical testing
- Detection of chlamydia and gonorrhoea in urine specimens
- >80% of US blood donations screened using TMA
- Methodological advantages - rapid, isothermal
- Ideal for rRNA amplification

Targeting ribosomal RNA:

- Higher copy number
- More sensitive
- Less risk of false negatives









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## Sample Collection and Handling

- Collect 100 ml water sample
- Filter sample through 0.45 μ membrane
- Wash to remove sample matrix







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## rRNA Extraction (1)

Cell lysis under mild conditions using combination of lytic enzymes and detergents at 37°C for 10 minutes





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## rRNA Extraction (2)

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### Magnetic Particle Processors

Automated 15 wells

Manual 100 tubes

Automated 96 wells

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- Filter sample (100 ml) → Transfer filter to lysis container → Add lysis solutions → Lyse bacteria on filter
- Take 1 ml lysate → Add magnetic particles → Magnetic separation
- Add rRNA target and amp reagents to 96 well plate → Read plate in engine

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Real-time amplification monitored by fluorescence using high-quality temperature-controlled fluorimeter.

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### Analytical Sensitivity

(C(T) = Emergence Time)

Assay Number	0 CFU (1/C(T))	10 CFU (1/C(T))
1	0.020	0.034
2	0.016	0.032
3	0.017	0.031
4	0.022	0.034
5	0.024	0.034
6	0.023	0.035
7	0.022	0.036
8	0.021	0.034
9	0.021	0.034

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### Reproducibility

Enterococcus log CFU	rRNA log fg/well
1.5	2.0
2.0	2.4
2.5	2.9
3.0	3.0

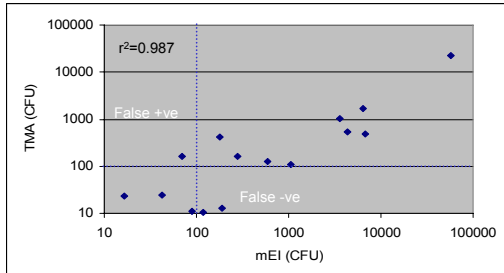
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### Dynamic Range

(C(T) = Emergence Time)

log CFU	C(T)
10	28.5
100	25.5
1000	22.5
10000	19.5

### Correlation with Standard Method



### TMA Analytical Specificity

Enterococcal Species	C(T)	Non-enterococcal Species	C(T)
<i>E. faecalis</i>	10.42	<i>Listeria monocytogenes</i>	>70
<i>E. gallinarium</i>	10.02	<i>Carnobacterium piscicola</i>	>70
<i>E. faecium</i>	9.81	<i>Lactobacillus caseii</i>	>70
<i>E. casseliflavus</i>	10.36	<i>Aerococcus viridans</i>	>70
<i>E. mundtii</i>	9.25	<i>Streptococcus bovis</i>	>70
<i>E. durans</i>	10.39	<i>Streptococcus equi</i>	>70
<i>E. hirae</i>	10.09		
<i>E. columbae</i>	13.50	(C(T) = Emergence Time)	
<i>E. avium</i>	>70	(>70 indicates negative result)	
<i>E. pseudoavium</i>	>70		
<i>E. malodoratus</i>	>70		
<i>E. raffinosus</i>	>70		
<i>E. dispar</i>	>70		
<i>E. saccharolyticus</i>	>70		

### Summary

It has often been proposed that nucleic acid based methods are capable of providing a more rapid means of detection of micro-organisms than conventional culture. Real-time transcription mediated amplification is a rapid, isothermal process which offers a highly sensitive and specific platform for the detection and quantitation of micro-organisms in environmental samples.