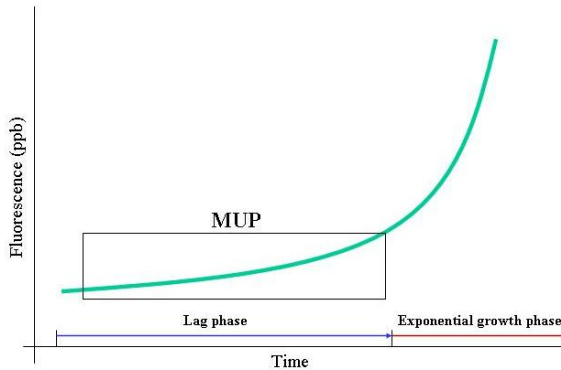


## Analysis Formats and Tests for C-ALARM, CALM, and CMD.

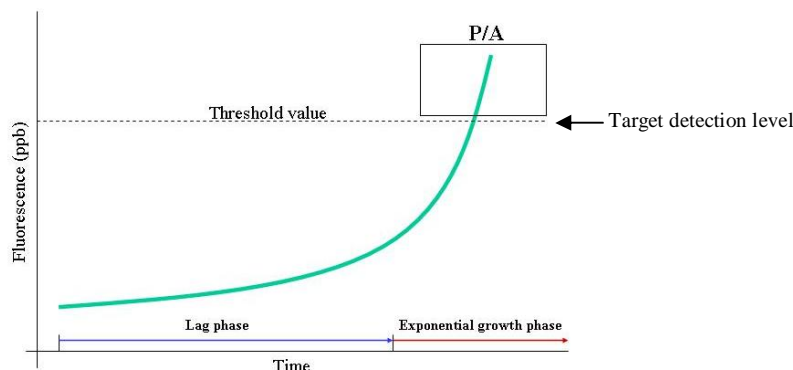
### ■ MUP (MU production) Early warning/screening test

Levels of bacteria	High (>500 cfu/100ml CMD)
Time to detect	2-3 hours
Measurement	Linear increase in fluorescence due to enzyme activity prior to bacterial growth. manual calculation (CMD)
Sample volume	5-10ml
Media	Colifast 6, Colifast <i>E.coli</i> . 5-10ml 2X concentration
Number of vials per sample	1 small vial 20ml volume
Highlights	<ul style="list-style-type: none"> <li>- Quick results, short incubation period</li> <li>- Wide range/unlimited number of possible results</li> </ul>
Comments	<ul style="list-style-type: none"> <li>- May need local calibration for each water source</li> <li>- Free enzymes and enzymes from other organisms may interfere</li> <li>- The results are based on the enzyme activity from both viable and dead bacteria</li> </ul>



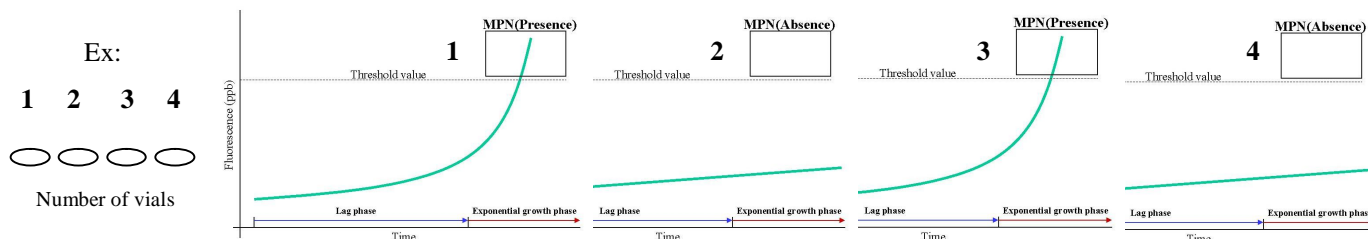
### ■ P/A (Presence/Absence) Detection of bacterial growth.

Levels of bacteria	Low (0-5 cfu/100ml)
Time to detect	10-14 hours
Measurement	Bacterial growth. Based on fluorescence above (presence) or below (absence) a threshold value.
Sample volume	100 ml: CMD: filtrated onto one 25mm filter, CALM: 4 X 25ml water sample, C-ALARM: 100 ml water sample.
Media	Colifast PA, Colifast <i>E.coli</i> , Colifast Milk, Colifast Pa* (bottled water). 10ml 1X concentration (CMD), 12.5 ml 3X concentration (CALM), 50 ml 3X concentration.
Number of vials per sample	1 small vial (CMD) 20 ml volume, 4 large (CALM) 40ml volume, 1 large (C-ALARM) 50 ml volume
Highlights	<ul style="list-style-type: none"> <li>- Based on the enzyme activity of viable bacteria</li> <li>- Robust method</li> <li>- High specificity</li> </ul>
Comments	<ul style="list-style-type: none"> <li>- Longer incubation period</li> <li>- Two possible results (CMD and C-ALARM). Five results CALM (4 vial MPN).</li> <li>- Manual filtration (CMD)</li> </ul>



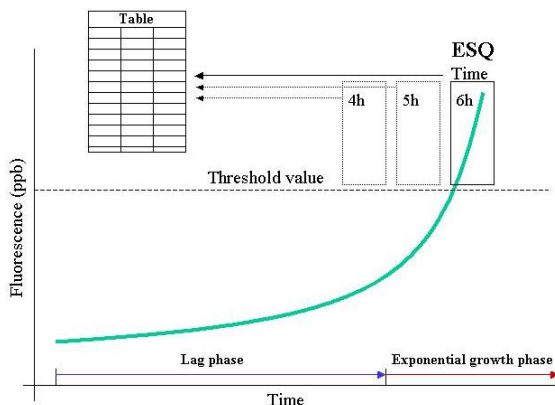
## ■ MPN (Most probable number, based on P/A) Statistical estimate of bacterial growth

Levels of bacteria	Low, medium (1-5000 cfu/100ml)
Time to detect	9-12 hours
Measurement	Bacterial growth. Based on number of positive vials (above threshold value) Automated MPN calculation CALM, manual calculation CMD.
Sample volume	0,05-200ml (depending on local bacterial level)
Media	Colifast 6, Colifast <i>E.coli</i> , Colifast Milk, Colifast Pa* (bottled water). From 10ml 1X concentration to 12.5 ml 3X concentration.
Number of vials per sample	5 (4-8) small 20 ml, volume or large 40 ml volume (depending on local bacterial level and target range of results)
Highlights	<ul style="list-style-type: none"> <li>- Based on the enzyme activity of vital bacteria</li> <li>- Robust method</li> <li>- High specificity</li> </ul>
Comments	<ul style="list-style-type: none"> <li>- Long incubation period</li> <li>- Defined range/limited number of possible results</li> </ul>



## ■ ESQ (Estimated Semi-Quantification) Estimated growth based on historical data

Levels of bacteria	Low to high (from 1cfu/25ml)
Time to detect	2-12 hours
Measurement	Bacterial growth. Time to detect. Based on time to reach threshold level linked to a semi-quantification table based on empirical data. Automated ESQ calculation CALM, manual calculation CMD
Sample volume	5-25ml (10-50, duplicate). Depending on local bacterial level.
Media	Colifast 6, Colifast <i>E.coli</i> , Colifast milk, colifast Pa* (bottled water). From 10 ml 1X concentration to 12.5ml 3X concentration
Number of vials per sample	1 small 20 ml volume or 1 large 40 ml volume (2, duplicate)
Highlights	<ul style="list-style-type: none"> <li>- Quick results when bacterial level is high</li> <li>- Based on the enzyme activity of vital bacteria</li> <li>- Many samples per run (CALM)</li> <li>- Wide range/ large number of possible results</li> <li>- High specificity</li> </ul>
Comments	<ul style="list-style-type: none"> <li>- Have to establish an ESQ table (site calibration)</li> <li>- Long incubation period when the bacterial level is low</li> </ul>



\* *Pseudomonas aeruginosa* (Colifast Pa medium)

Type of water	Bacterial level	Analysis format
Bottled water *	Low (0 cfu/100ml)	P/A, MPN
Raw water Clean	Low (0-5 cfu/100ml)	P/A, MPN
Raw water Medium	Medium (5-1000 cfu/100ml)	MPN, ESQ
Raw water Dirty	High (> 500 cfu/100ml)	MPN, MUP, ESQ
Effluent/waste water	High, medium (> 100 cfu/100ml)	ESQ, MUP
Recreation water/fresh water/sea water	Medium (< 500 > cfu/100ml)	MPN
Environmental water (fresh water)	Medium (< 500 > cfu/100ml)	MPN, ESQ, MUP
Distribution/finished water (non-disinfected)	Low (0 cfu/100ml)	P/A, MPN
Industrial process water	Low to high	All (on level)