CHALLENGES AND FUTURE FORECASTS OF MEASUREMENT TECHNOLOGIES IN WATER MICROBIOLOGY

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### AREAS OF WATER MICROBIOLOGY RESEARCH

<table>
<thead>
<tr>
<th>Natural Waters</th>
<th>Drinking and Household Waters</th>
<th>Industrial Waters</th>
<th>Recreational Waters</th>
<th>Waste Waters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lakes</td>
<td>Tap waters</td>
<td>Food industry</td>
<td>Swimming pools</td>
<td>Residential wastewaters</td>
</tr>
<tr>
<td>Rivers</td>
<td>Bottled waters</td>
<td>Pharmaceuticals</td>
<td>Jacuzzis</td>
<td>Industrial wastewaterst</td>
</tr>
<tr>
<td>Run-off water</td>
<td>Water machines and dispensers</td>
<td>Cosmetic industry</td>
<td>Water parks</td>
<td>Mining effluents</td>
</tr>
<tr>
<td>Ground water</td>
<td>Storage of drinking water</td>
<td>Chemical industry</td>
<td>Spas</td>
<td>Hospital wastewaters</td>
</tr>
<tr>
<td>Wet deposits</td>
<td></td>
<td>Forest industry</td>
<td></td>
<td>Agricultural load</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fish farming</td>
<td></td>
<td>Waste water load from the environment</td>
</tr>
</tbody>
</table>

The water content of the products, wash waters etc.

### DISTRIBUTION OF INFECTIONS, EPIDEMICS AND ANTIBIOTIC-RESISTANT MICROBIAL STRAINS SHALL BE PREVENTED

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STANDARD DEVELOPMENT IN WATER MICROBIOLOGY


Laboratory activities of social and health care administration are based on either national laws or regulations or international agreements, which must be met (Report by the laboratory workshop of Social and Health Care Administration. STM 31.10.2008).

THREATS ARE DIVERSE, NOVEL METHODS ARE NEEDED!

• EPIDEMICS, LONG-RANGE CARRYOVER
• SPOILAGE OF TAP WATER NETWORK SYSTEMS
• NEW PATHOGENS, DRUG RESISTANCE
INDICATOR BACTERIA
• are bacteria which can be found downstream from the sources of very diverse types of pollution
• are easy to enrich, cultivate, count and handle

Coliforms and Enterococci are common bacteria living in the intestines of human beings and warm-blooded animals. They are used as indicators of faecal contamination. Indicator bacteria can be found also from the natural environments without faecal load. Advanced analytics is therefore needed to trace the source of contamination.

PMEU technology helps to detect indicator bacteria from samples where their counts are low or they are in bad condition. It is also a tracing tool whenever the origin of the microbes shall be found ("source-tracking").
APPLICATION OF INDICATOR BACTERIA:

- *Escherichia coli*
- Enterococci
- Total count of coliforms
- *Pseudomonas aeruginosa, Clostridium perfringens*

TOTAL COUNT OF HETEROTROPHIC BACTERIA

INDICATION OF PATHOGENS

- *Salmonella*
- *Campylobacter*
- VIROLOGIC ANALYSES
TRADITIONAL CULTIVATION METHODS

- Membrane filtration
- Plate cultures
- MPN ("Most Probable Number") broth tubes
- Colilert®

ACCELERATED ENRICHMENT CULTIVATION (PMEU)

METHODS OF MOLECULAR BIOLOGY

- immunoassays
- PCR
- biosensors, chips

TESTING METHODS IN WATER HYGIENE

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**COMBINATION OF PMEU ENRICHMENT METHOD AND COLILERT™ BROTH**

*E.coli* (three syringes in the middle) growing in Colilert™ broth where yellow color is the indicator for all coliforms

Fluorescence of three *E.coli* cultures, when illuminated with UV, in Colilert™ broth; this fluorescence is a specified indication of only *E. Coli*; other bacteria are non-fluorescent

CHROMOGENIC NUTRIENT MEDIA (ChromAGAR TM plates, Becton Dickinson Inc (Invented by Alain Rambach)) ARE ONE ALTERNATIVE FOR RAPID IDENTIFICATION

E.coli
Klebsiella mobilis
Enterococcus sp.
Bacillus cereus
DETECTION OF MICROBES IS A CHAIN PROCEDURE

SAMPLING

TRANSPORT + PRE-ENRICHMENT

ENRICHMENT CULTIVATION

DETECTION

ANALYSIS

## DECENTRALIZED (REMOTE) CONTROL, CENTRALIZED RESULTS

<table>
<thead>
<tr>
<th>Sample point</th>
<th>Total plate count cfu/100 ml</th>
<th>coliformic bacteria cfu/100 ml</th>
<th>E.coli cfu/100 ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>2 days</td>
<td>6 days</td>
</tr>
<tr>
<td>Sample point 1</td>
<td>0</td>
<td>2000</td>
<td>6000</td>
</tr>
<tr>
<td>Sample point 2</td>
<td>0</td>
<td>full</td>
<td>full</td>
</tr>
<tr>
<td>Sample point 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample point 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample point 5</td>
<td>0</td>
<td>10000</td>
<td>10000</td>
</tr>
<tr>
<td>Sample point 6</td>
<td>0</td>
<td>0</td>
<td>10000</td>
</tr>
<tr>
<td>Sample point 7</td>
<td>0</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td>Raw water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lake water</td>
<td>full</td>
<td>full</td>
<td>full</td>
</tr>
<tr>
<td>Purified water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Bacteriological mapping of tap water network (Hakalehto et al. 2010)**

**Share of Enterocci types in lake water samples (Heitto et al. 2009)**
POLARIS – national project for the development of total water quality control in Finland

• Basics: Current systems of water quality measurements cannot detect the spoilage of ground water. In most cases, contamination of tap water will be revealed only when first illnesses have been detected.

• Implementation: Risk assessment, ON LINE measurement technologies, effective modeling-supported picture of the situation and process optimization will all be applied into the project.

• Six Finnish water research institutes, Finnish Water and Waste Water Works Association (FIWA), 13 enterprises and three Finnish waterworks are joining this research project.
PMEU technology in POLARIS project

• Enrichment of the samples with PMEU equipment enhances and speeds up the detection of bacteria
  • **Application of** Colilert TM broth in rapid coliform and *E.coli* detection in PMEU
  • Real-time monitoring of the bacterial growth via Internet
  • Recovery of environmental stress-suffering hygiene indicators and heterotrophs

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PMEU technology in POLARIS project

PMEU Tent

PMEU Tent w/net connection

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Photos: Kevin King
PMEU acting in water works tests of Polaris project. Nutrient broth: Colilert TM. Reference analyses with traditional methods were performed in the Kuopio laboratory of THL / Dep. Of Water and Health. Every partner of this project has been able to follow the real-time results (growth curves) from PMEU via Internet. Next step of Polaris project includes testings of ASCS (Automated Sample Collection System) by SAMPLION Ltd. in water works environment and in tap water distribution network.

Photo: Kevin King.
PMEU technology in POLARIS project

Identification of *E. coli* (1st from left) with UV in Colilert™ medium

Next: results from current field trials of Polaris project.
Sample from a ground water well. Total volume of 100 ml divided into two syringes. An example of clean water: no growth in 24 h

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Sample from a ground water well B. Total volume of 100 ml divided into two syringes. Growth was detected in one syringe in less than 20 h. Density of indicator bacterium in the beginning of the analysis was appr. 1 cell/100 ml.

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Sample from a ground water well C. Total volume of 100 ml divided into two syringes. Atypical growth, beginning after 14-16 hours from the start of the analysis.

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Microbial growth in replicate samples of lake water after 10 h from the beginning of the analysis.

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Syringes No. 5 contained $10^5$ *E.coli* cells/ml in the beginning of the test. Final density of *E.coli* in these syringes was $10^8$ cells/ml. The values of *E.coli* densities in syringes No.1 were 2-4 cells/ml and $10^6$ cells/ml, respectively. Incubation temperature: +37°C.
Campylobacter coli ANALYSES WITH PMEU METHOD
(Laboratory of FINNOFLAG Ltd., 2005)

- Campylobacter analyses with PMEU in 2005 (Campylobacter coli)

- The uppermost curve shows the result of an enrichment cultivation where bacterial density was even 100,000,000,000 cells/ml ($10^{11}$) at the end of the analysis

- Increased speed of the analysis with PMEU method is also visible

- The validation of PMEU Campylobacter method, performed in cooperation with THL (prev. KTL), was based on these preceding tests

- Results of this scientific research have been reported in 2009 (Canadian Journal of Microbiology)
Enrichment and detection of *Salmonella sp.*, inoculated in a tap water sample, with portable PMEU Scentrion R unit (manuscript in press; Hakalehto et al. 2010). These cultures have started from one cfu/ml (colony forming unit) of salmonella in the tap water sample. The analytical response in less than 9 hours can be seen as the drop of the detector charge, caused by the gaseous metabolites of the culture.
Gaseous emissions, produced by the metabolism of water indicator bacteria in PMEU Scentrion® cultures

- Identification of bacterial contaminants
Environmental spread of paper industry waste waters can be followed with bacteriological mapping

Upper row: 48 h cultivation without pre-enrichment

Lower row: pre-enrichment in PMEU (6 h) + 24 h cultivation on DryCult™ medium (Orion Diagnostica)

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CHALLENGES OF MEASUREMENT TECHNIQUES IN WATER MICROBIOLOGY

• DEVELOPMENT OF SAMPLING PROCEDURE FOR BOTH CONTINUOUS MONITORING (ASCS) AND CONVENTIONAL CONTROL

• COMBINED DETECTION OF INDICATOR BACTERIA, PATHOGENIC MICROORGANISMS AND TOTAL LOAD OF HETEROTHROPHIC BACTERIA

• DEVELOPMENT OF REMOTE CONTROL OF WATER QUALITY AND EARLY-WARNING SYSTEM

• IDENTIFICATION OF NEW THREADS (INCL. STRAINS WITH ANTIBIOTIC RESISTANCES)

• INVESTIGATIONS OF THE LOAD ORIGIN (”SOURCE-TRACKING”)

• DETECTION OF MICROBES SUFFERING FROM ENVIRONMENTAL STRESS AND/OR TO BE PRESENTED IN SMALL QUANTITIES

• CONTINUOUS DEVELOPMENT OF METHODS IS A NECESSITY

A SINGLE CELL, VIRUS OR VIRUS PARTICLE IS A POTENTIAL HAZARD!

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Literature


Wirtanen, G. and Salo, S. 2010. PMEU-laitteen validointi koliformeilla. (Report VTT-S-01705-10) VTT Expert Services Oy, Espoo, Finland