Current Issues and Approaches to Microbial Testing of Water: Applicability and Use of Current Tests in the Developing World

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Status of Microbial Water Testing in the Developing World

- Most drinking waters are never tested
- Tests are not accessible, too complicated and too costly
- Waters that are tested are tested rarely and often poorly
  - Often tested only at a treatment works and not at taps
- Microbial water test results are not used for rational or timely decision making for water safety
- Microbial water testing is not done for the right reasons or within a health risk-based framework
  - End-product analysis after water treatment
  - Not used in risk assessments for water supplies
  - Not used to support Water Safety Plans (management)

Drinking Water Quality and the MDGs
'Improved' ≠ 'Safe' Water

- Drinking water quality is not systematically addressed for water policy and management through the current JMP of the MGD effort
  - Little or no testing of source or drinking waters
- No objective assessment of which sources are 'safe'
- JMP (WHO & UNICEF) uses improved/unimproved classifications of sources as proxy for safe/unsafe water.
  - NOT PREDICTIVE OF HEALTH RISK!
  - Improve water is often microbially contaminated and unsafe
- Need data for:
  - Source classification and selection
  - Choice and effectiveness of treatment
  - Drinking water quality as an element of Water Safety Plans

Diagnostic Tests for the Developing World:
We Can and Should Apply this to Water Tests!

- **A**ffordable by those at risk
- **S**ensitive (low false-negatives)
- **S**pecific (few false positives)
- **U**ser-friendly (simple to perform)
- **R**apid and robust; easy to store
- **E**quipment-free (ideally no electricity)
- **D**elivered to those who need it

(Urdea et al 'Requirements for high impact diagnostics in the developing world' *Nature S1*, 73-79 (23 Nov 06)

Issues in Water Testing

- Why test water? What will you/we do with the results?
- Who has the data and what will they do with them?
  - How will the results be used in a beneficial way?
- Target analytes: microbes!
  - Main microbial targets: detect/quantify fecal contamination
  - Chemicals only in certain high risk settings; As & Fl
- Test basis, analytical method and format
- Test location and conditions: lab or field?
- Test readout and interpretation: P-A or quantitative?
- Test performers and user groups: decision-based testing
- Ease of use, number steps, skill level
- Supporting infrastructure; portability; electricity, sterility…
- Cost
- Accessibility, supply chain for consumables, etc.
- Production, fabrication, marketing and distribution

Current Options for Microbial Testing Venues

- Static laboratories
  - Expensive facilities and equipment, high overhead, consumables and high calibre staff
  - Long chain to get samples from field to lab
- Field labs and portable labs

  Cheaper, but still expensive, supply chain issues and need for trained staff
- Disposable tests in the field
  - Petrifilm (cold chain issue)
  - Easygel plates for colony counts (cold chain issue)
  - Presence/absence by H_2S
  - Others
  - All are now too expensive and current access is limited

Desirable Goals for Microbial Testing

- Portable, self-contained, lab-free, electricity-free
- Low cost:
  - goal: $0.1 per test
  - globally available and accessible
- Access:
  - multiple marketing/distribution options
  - full cost recovery + profit
  - humanitarian distribution w/ subsidized or no user cost
- Data Communication:
  - Save, convey and use the data for decisions!
- Education
  - Use water testing to educate and mobilize all stakeholders - especially youth

Approaches and Options for Microbial Testing

- Microbe(s): *E. coli – the* “universal” drinking water indicator
  - Other indicators and even pathogens deserve consideration
- Basis/Method: Now: culture with visual readout
  - Future:
    - Culture with enhanced detector and direct detection
    - Non-culture methods: molecular, chemical, other emerging techs.
- Formats: Liquid culture, multiple volumes, decimal bands
  - Alternatives: colonies or plaques; absorbent pads, membrane filters, ambient gel media for pour plates, etc.
    - dispensing and format issues
- Location/Conditions: non-lab (field); ambient temperature or internal temperature control within the test system
- Readout/interpretation/Ease of Use: Visual examination, probably color change; counts only; no language needs

Current Options for *E. coli* Tests: Applying Developed Country Culture Methods in Developing Countries - Most are Impractical, Cumbersome and Expensive!

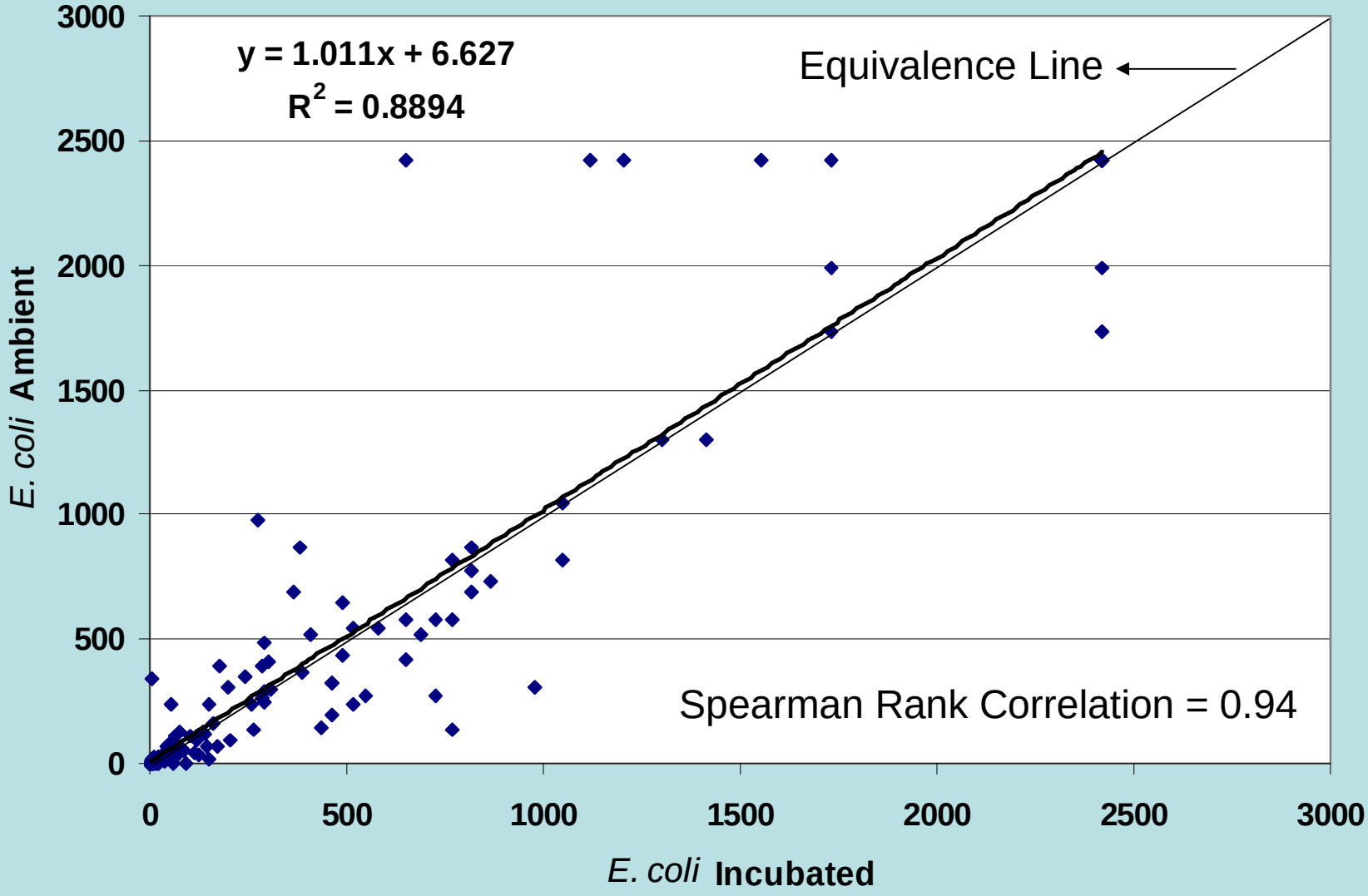
- Liquid quantal (MPN; but not P-A!) assays:
  - Colilert (defined substrate technology)
  - Other chromogenic/fluorogenic broths with **β**-glucuronide substrates for *E. coli*
  - Less specific broths followed by biochemical confirmation (e.g., urease test)
- Membrane filter methods:
  - Chromogenic/fluorogenic *E. coli* media
- Dip cells: hold only small sample volumes
- Pour plates with pectin gel media:
  - (Coliscan Easygel)
- PetriFilm and other films; as culture plates

Current Options for *E. coli* Testing in Developing Countries – Culture and Incubation Conditions

1st Principle: Substrate specificity trumps temperature!

- Ambient temperatures in tropical climates are typically >25-40+ ^{o}C
  - *E. coli* and other enteric bacteria grow well in this range
  - Good growth occurs; not just at 35-37 or 44.5 ^{o}C
  - *E. coli* detection specificity can be achieved by hydrolysis of a specific (β-glucuronide) biochemical substrate giving a colored or fluorescing product in medium or colony
  - masking, interference, false + by other bacteria?
- Petri films, other absorbent pads, dip cells, MF plates and small volumes of liquid cultures can be incubated on one's body
  - In a pocket, under the arm, etc.
- Exothermic chemicals to maintain temperature: "warmers"

Colilert *E. coli* MPNs in Dominican Republic Village Water by Standard and Ambient Temperature Incubation

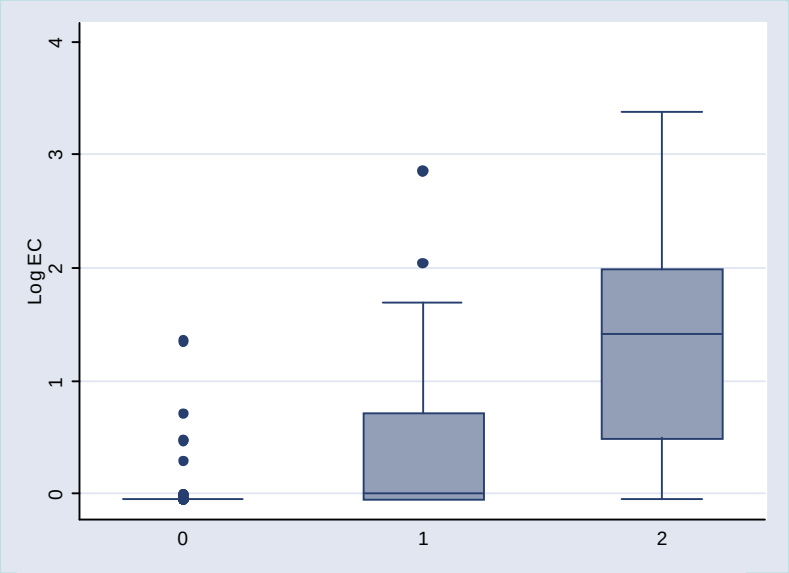


Other Tests That are Simple and Informative
The Hydrogen Sulphide-Producing Bacteria (H_2S) Test

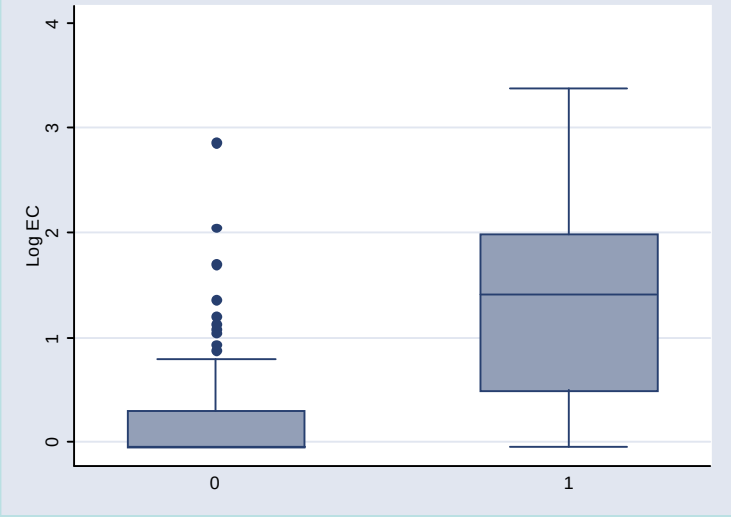
- Many (but not all) H_2S-producing bacteria are of fecal origin
  - Some coliforms/FC, *Salmonella* spp., *Proteus* spp., *Clostridium perfringens,* others
- H_2S-producing bacteria can be assayed by simple tests using inexpensive, stable and easily made media
- Many studies indicate good correlations with fecal indicator bacteria and *Salmonella* spp.
- BUT:
- Usually Presence-Absence; not quantitative
- No epidemiological data on relationship between H_2S bacteria levels and waterborne illness risks
- Can be easily adapted to a quantitative test format

- Further details: Sobsey, M. and F. Pfaender (2002) Evaluation of the H2S Method for the Detection of Fecal Contamination of Drinking-water. WHO/SDE/WSH/02.08, World Health Organization, Geneva (http://www.who.int/water_sanitation_health/dwq/WSH02.08.pdf)

Relationships between *E. coli* Colilert MPN and H_2S Levels as 2 & 3-Level Decimal Bands in DR Village Waters



H_2S/E. coli→ ↓	0	1	2	Total
0	39 (80%)	9 (18%)	1 (2%)	49
1	19 (44%)	17 (40%)	7 (16%)	43
2	36 (10%)	97 (28%)	220 (62%)	353
Total	94	123	228	445



H_2S/E. coli → ↓	0 (0 – 9)	1 (10+)
0 (0-~10)	84 (91%)	8 (9%)
1 (>10)	134 (38%)	220 (62%)

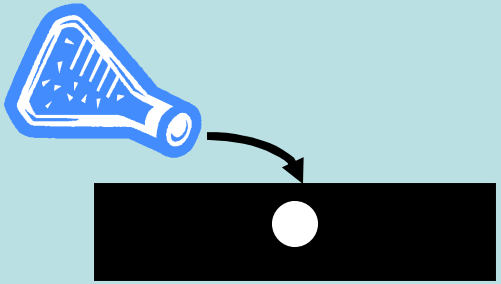
Coliphage Culture Detection in 2-3 Hours

Sample + medium + *E. coli* host

Can do in multiple volume format for quantal MPN results

Incubate 3 hours for replication

Remove small volume to detect coliphage antigens by particle agglutination – 1 minute



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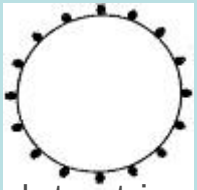
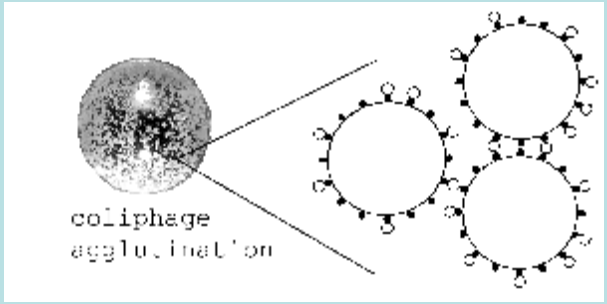


detection solution

+

coliphage



Existing Microbial Tests: Summary and Directions

- ***E. coli* target: many test options and resources available for use in developing countries**
  - **Alternative test formats and media**
  - **Simple, low cost, local materials available**
  - **Ambient temperature incubation works well for some *E. coli* tests**
- **H_2S bacteria test is promising for at least screening**
  - **Conservatively good agreement with *E. coli* results**
  - **Need to quantitatively compare to *E. coli*health outcomes**
  - **Simple coliphage methods are a promising option**
- **Further evaluate candidate methods and materials in different developing countries and settings**
- **Inform stakeholders of forthcoming test options**
- **Communication, marketing and outreach**

AQUATEST PROJECT:

A study to develop low cost, accessible and affordable tests for fecal contamination of water in the developing world

Stephen Gundry, Project Principal Investigator
University of Bristol, Bristol, UK
Water and Environmental Management Research Centre
and the AQUATEST Study Team, multiple organizations

AQUATEST Concept

- Low cost
  - Target manufacturing cost: USD 0.10 per test
- Low skill
  - Zero training - e.g., home pregnancy or glucose test
- Better than P/A
  - Not full enumeration of *E. coli*
  - Bands of water quality (order of magnitude): ‘traffic light’
- Links
  - Water Safety Plans
    - WHO health risk-based water quality management system
  - Risk monitoring systems not compliance

Uses of AQUATEST

- Monitoring
  - Relative risk monitoring and response
  - Measuring MDG progress
  - Ad-hoc surveys (including disasters)

- People power !
  - Communities take responsibility
  - Households react: better water management
  - Hygiene educational benefit

Who will use it?

- Professionals
  - Environmental health officers
  - W&S commissioning engineers
  - Survey technicians (disasters)
- Water consumers
  - Non-specialist staff (e.g. clinic nurse)
  - Community leaders
  - Householders

AQUATEST I

- Start date: 1 July 2006 (but…….funding delay)
- 12 month preparatory study to establish:
  - needs
  - technological feasibility
  - funding requirements
- Mid-2007: full R&D proposal
- Funding Euro 446,000 by European Union under FP6: Global change and Ecosystems
- 13 participants (Europe 5; Developing countries 3 USA 3; and International organisations 3)

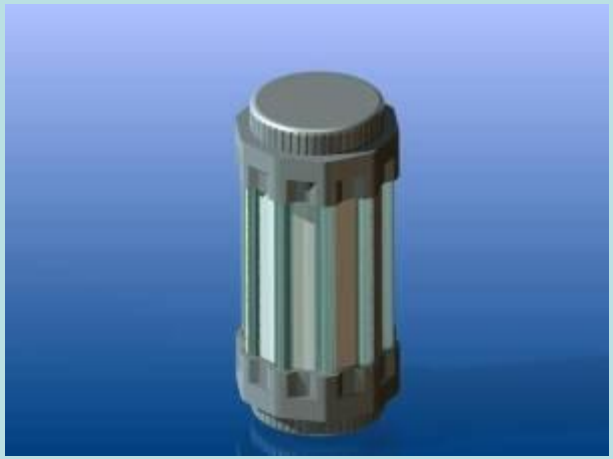
AQUATEST II ($13+ Million, Gates Fdn.) - Objectives

Deliver a low-cost water test for wide use in developing countries, with a sustainable basis for its manufacture, distribution and marketing; 4 yrs.

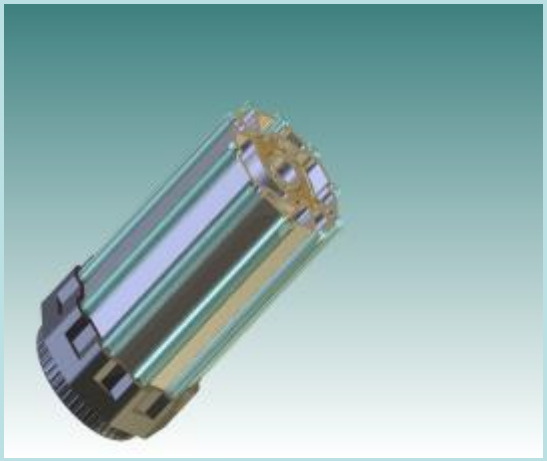
- I. Develop (2 yrs.) & test (4 yrs.) a simple-to-use diagnostic for microbiological water quality (i) based on chromogenic *E. coli* detection; (ii) manufactured at a cost of $0.10 ea. in developing countries; (iii) that can incorporate technology/microbial advances.
- II. Get end-user input (2 yrs) to inform Aquatest design and marketing strategies, (ii) test (3 yrs) acceptability among professional users; survey behavior change in response to test use (iii) trial (4 yrs.) self-testing among communities and households to determine impact on water management behavior and estimate water quality improvements, wellbeing, health and economic conditions.
- III. Analyze (2 yrs.) two principal markets and prepare (4 yrs.) generic licensing, manufacturing, distribution and marketing plans,
- IV. Produce (4 yrs.) an action plan to support and promote affordable water testing in developing countries based on (i) estimated benefits from widespread adoption in two markets (i.e. India and South Africa); (ii) a template to report test results to stakeholders using cell phone technology; (iii) analyze applications for low cost water testing; (iv) recommend necessary changes in policy, regulation and governance to support uptake; and (v) promote low cost testing through targeted international activities.

AQUATEST II:
DEVICE DESIGN and USE FEATURES

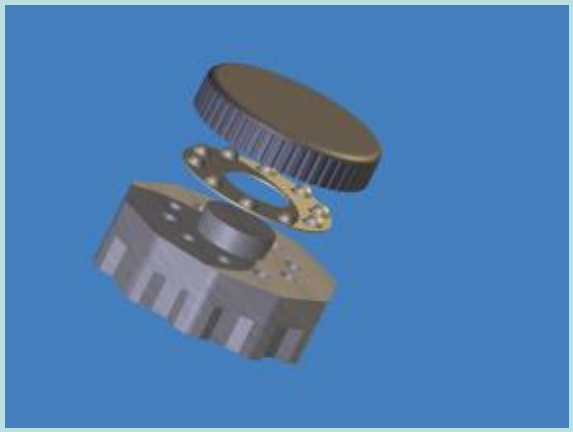
Prototype: solid model



Device assembly

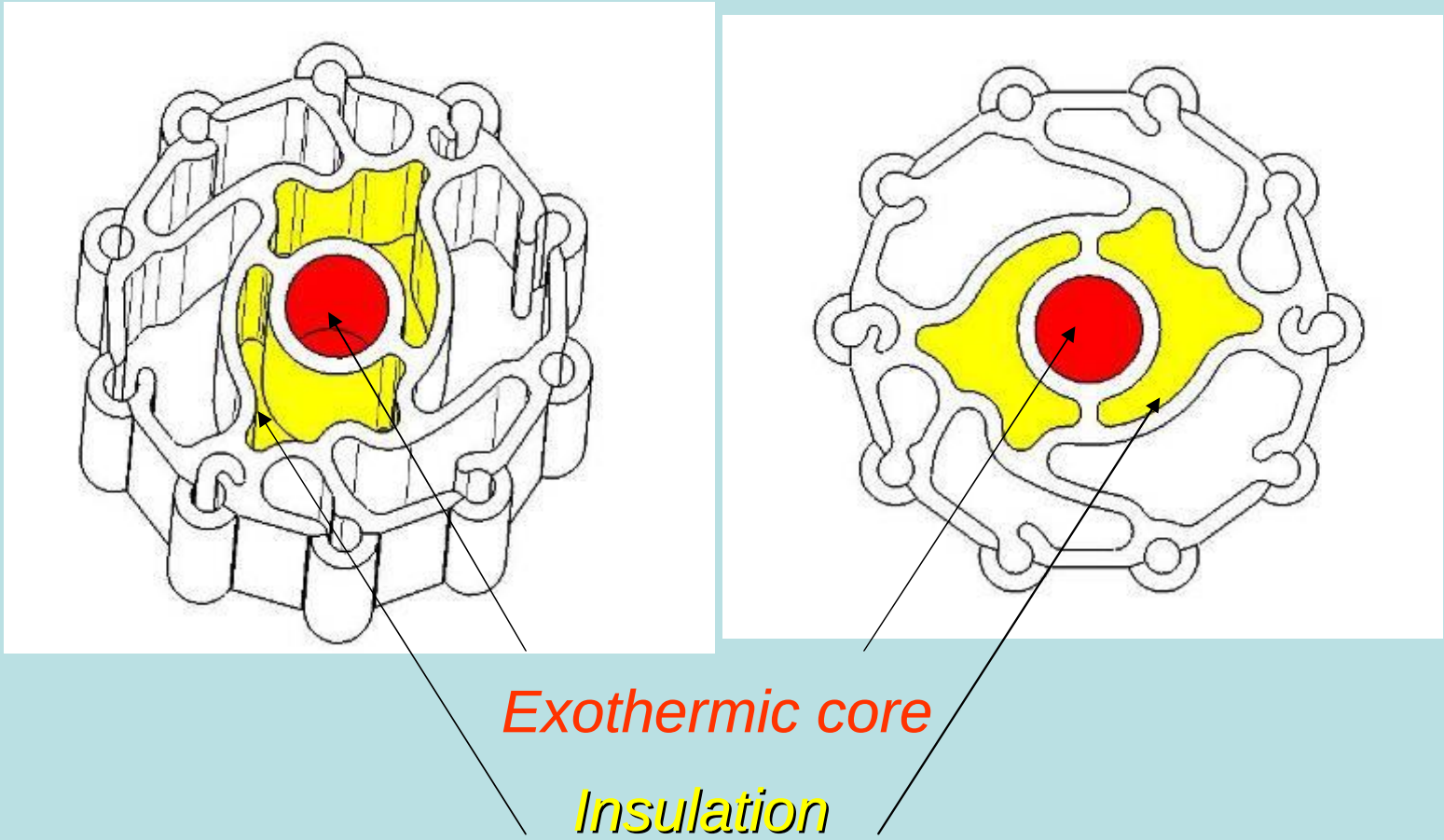


Device Assembly - lid

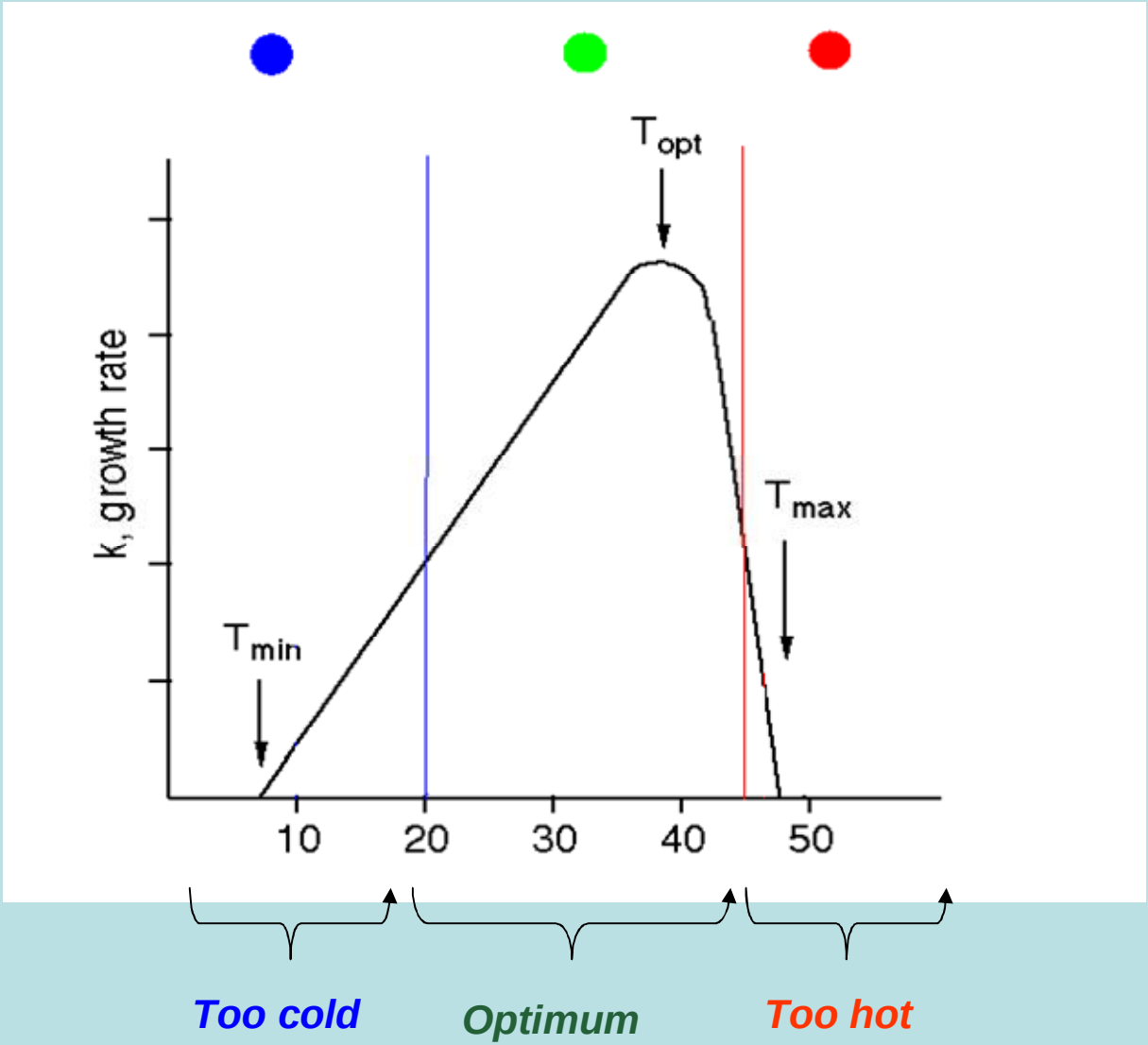


- Device lid contains tablet or granular *E. coli* medium
- Release medium into sample, dissolve, incubate
- Candidate medium is *E. coli*-specific chromogenic Beta-glucuronide medium, existing or newly developed
- Incubate and read results as color bands of quality
- Link to cell-phone enabled data logging and reporting system to local, national and global databases

Internal design: self-incubating version?



Self-incubating device: temperature warning to users



AQUATEST II: Proposed Development Phases

- Initial: culture-based tests with overnight incubation
  - Chromogenic media for *E. coli* or other target microbes
- Later: Culture based tests with same-day results
  - Use optical devices for sensitive detection of color or fluorescence
- Maybe eventually: brief or no culture step with detection of *E. coli* –specific (other target microbe-specific) target macromolecule or activity by genetic, antigenic or other analytical methods
  - Biosensors
  - Microfluidics
  - Optical sensors
  - Others

Methods Beyond AQUATEST: Non-Culture Methods for *E. coli* and Other Microbes

- Immunoassays (e.g., Watersafe® Bacteria Test)
  - Poor sensitivity
  - No indication of viability
  - False positives
  - Couple to culture or other target enrichment
- Other non-nucleic acid methods
  - MALDI-TOF-MS detection
  - Specific ligand-binding and reporter molecules
- Nucleic acid methods
  - PCR, RT-PCR, NASBA, Microarrays, etc
- Biosensors for microbe-specific activities and their target macromolecules
  - Detection facilitated by microfluidics and advanced optical systems

Beyond AQUATEST- Recommendations

- Need greater efforts by more “players”
- Explore more test formats
- Explore alternative target microbes
- Engage water various policy and program sectors in how testing results would be used
  - Water science and engineering
  - Health
  - Development
- Link to waterborne disease epidemiology and to quantitative microbial risk assessment
- Educate! A tool for motivation, action and policy