Detecting coliform bacteria

Like E.Coli and other water contaminants

Main steps for bacteria detection

- Sample collection and concentration (Emilie)
- Capture of organism (PCR + surface recognition, enzyme/ substrate or nucleic a. detection methods)
- Detection (fluorometry, cytometry, electrochemical signal, …)
- Data transfer and real-time access

Capture

- Target is either removed, tagged or amplified
- This step is responsible for the selectivity of the approach
- It has three classes of methods:
  - Surface & whole-cell recognition
  - Nucleic acid
  - Enzyme/substrate

Surface and whole-cell recognition methods

- Include immunoassays, molecule-specific probes and bacteriophages
- They are potentially more rapid and sensitive, as well as adaptable to a wide class of indicators/pathogens
- A good point is that captured bacteria are still viable for further studies

Nucleic acid methods

- Include PCR, RT-PCR, q-PCR, NASBA and microarrays
- With microarrays, thousands of microorganisms can be analyzed at once
- NASBA is an isothermal method, which is like PCR, but with increased portability
- The most versatile, but they measure nonviable structures

NASBA = nucleic acid sequence based amplification

Enzyme/substrate methods

- Use fluorogenic and chromogenic substrates
- Enhancement of currently approved methods that lead to commercial kit creation (Colilert® form IDEXX)

Detection methods

- Optical: most frequent, but susceptible to turbidity interference
  - Spectrometers/fluorometers (portable, rapid, low-cost)
  - Flow cytometry (not portable or robust, need advanced training)
  - Fiber optics (sensitive, ability to make remote *in situ* measures)
  - Laser-based interferometry (high sensitivity down to 1 cell, used to detect contaminants in soil, groundwater)
- Electrochemical (fast & low-cost, have low detection limits but can be perturbed by seawater; use conductance, potential and voltage changes)
  - *E. coli method based on hydrolysis of 4-APGal to 4-aminophenyl (4AP)* → detection limit still>10^4 cfu/100ml that is too high for recreational water
- Piezoelectric (sensitivity levels have not been demonstrated at 1 cell/ml in 2005)

Limitations

- Capture
  - Antibody specificity
  - Temperature dependence of reactions
  - Live vs. dead, infective vs. inactivated
- Detection
  - Fluid streams
  - Temperature related stability
- Cost and technical experience required

Newly developed technologies

Portable, user-friendly devices for bacteria detection

Paper test strip

- self-contained, portable, bioactive paper sensor
- rapid (sec to min) and sensitive (~5cfu/ml) detection
  - However, to detect 1cfu/ml culturing time-consuming steps are required (~8h)
- based on intra-cell enzyme activity (GUS and/or B-GAL)

GUS: beta-glucuronidase, B-GAL: beta-galactosidase
cfu: colony-forming units

Portable sensor

- “embedded system that is highly competitive with SPC in terms of measuring time (3 – 12 hours depending on the sample contamination) and features user-friendly procedures, with no need of a laboratory environment, detects bacterial concentration in liquid and semi-liquid samples by using the impedance technique” (Grossi & al., 2013)

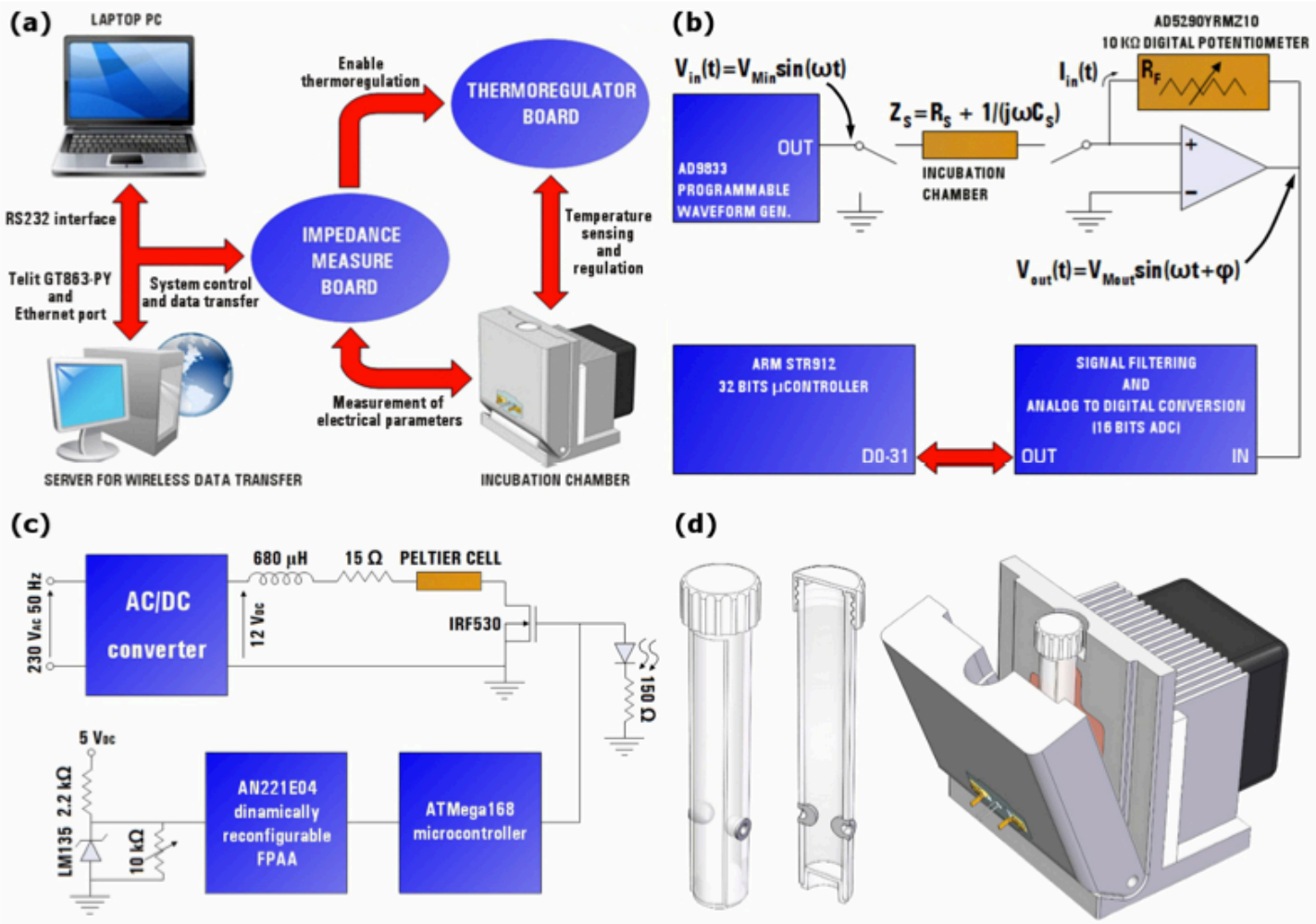

Fig. 1 Schematic representation of the sensor system (a), the circuit used to measure the sample electrical parameters (b), the thermoregulation circuit (c) and the incubation chamber (d).

Portable sensor

- “By using the appropriate enriched medium (enriched Lactose Broth and Lauria Bertani showed good results) either coliforms or total bacterial concentration can be reliably estimated with response time as low as 3 hours for highly contaminated samples (> 106 CFU/ml).” (Grossi & al., 2013)

LED-based portable fluorescence device

- "The application of an LED-based portable fluorescence device has the potential to achieve instantaneous results for the microbial load of potable water at modest cost and using minimal equipment and consumables, without waiting for results from sample culturing, thus potentially meeting the WHO requirements for more frequent, less sophisticated testing in the field rather than irregular and infrequent, but more comprehensive, tests. "(S. Cumberland & al., 2012)

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