

Development of an automated legionella detection system

Jan Consbruch

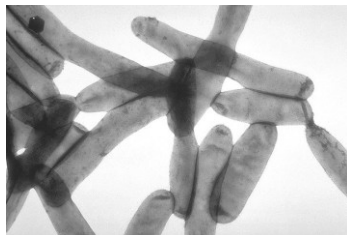
Overview

- Legionella pneumophila
- Classic analysis
- Automated analysis

Legionella pneumophila

-Conditions of living

- Prefers temperatures between 25°C and 50°C
- Sweet and salt water
- Standing and slow moving water reservoirs
- Lives in amoebae if available

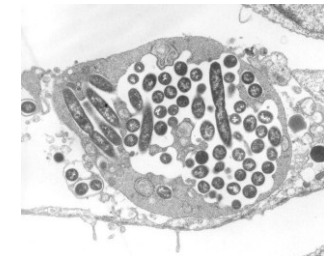


Source: <http://de.wikipedia.org/wiki/Legionellaceae>

Legionella pneumophila

-Disease

- Need to reach deeper lung region for infection
- Drinking riddled water not dangerous for healthy people
- Legionnaire's disease
 - 6.000 – 12.000 Infections per year (Germany)
 - 20% - 35% morality
- Pontiac fever
 - 1.000.000 Infections per year (Germany)
 - Heals in one week



http://de.wikipedia.org/wiki/Legionella_pneumophila

Classic analysis

- Take a sample of water
- Fishing out the legionella agents
- Amplify the number of agents by:
 - PCR
 - Cultivation
- Measure the total count
 - Fluorescent measurement
 - Count the cultivated colonies



Source: <http://www.rhein-berg-online.ksta.de>

Classic analysis -Cultivation

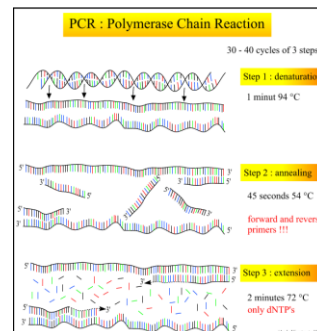
- Smear test on substrate
- Increase in Incubator
- Qualified staff
- Appropriated laboratory (C2)
- Duration time 1 – 3 days



Source: <http://en.wikipedia.org/wiki/Legionella>

Classic analysis -PCR

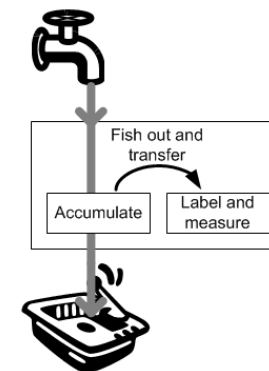
- Faster than cultivation (2 – 4 h)
- Complex sample preparation
- Qualified staff
- Appropriated laboratory (C2)
- Expensive equipment needed



<http://www.nymphenburger-gymnasium.de/gkbio/gentech/gentech.html>

Automated analysis

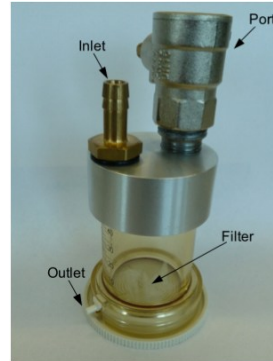
- Accumulate all agents
- Fish out the legionella agents
- Labeling the legionella agents
- Measure the amount of agents



Automated analysis

-Accumulate the legionella agents

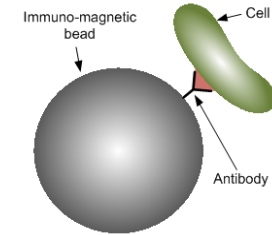
- Connect device with water supply
- Filter e.g. 100 l water
- Disconnect the water supply
- Open the port for further process steps



Automated analysis

-Fish out the legionella agents

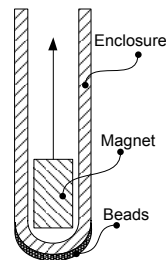
- Add immuno-magnetic bead
- Steer for about 30 minutes
- Selective antibodies bound to legionella agents



Automated analysis

-Fish out the legionella agents

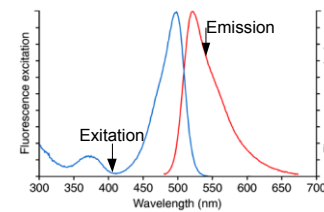
- Put magnetic finger into the cell
- Steer for about 30 minutes
- Move finger into analysis vessel
- Remove magnet



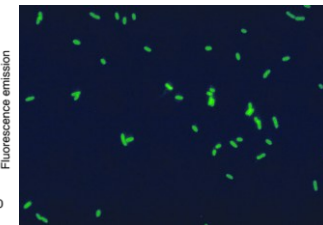
Automated analysis

-Labeling the legionella agents

- Add fluorescent dyes (SYBR green)
- Dye is only active if bounded to DNA



Source: www.invitrogen.com

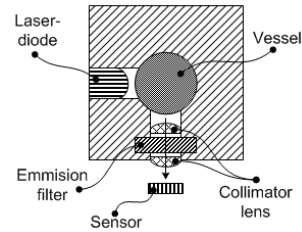


Source: http://de.wikipedia.org/wiki/Legionella_pneumophila

Automated analysis

-Measure the amount of agents

- Excite with blue light (405 nm)
- Filter out the emitted wave length shorter than 488 nm
- Measure the emission (522 nm)



Summary

- **Legionella**
 - Conditions of living
 - Disease
- **Classic analysis**
 - Cultivation
 - PCR
- **Automated analysis**
 - Accumulation of agents
 - Fishing out of legionella
 - Labeling
 - Measurement

Thanks for you attention!

Impressum

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