

Distinguishing living and dead bacteria by PCR: the Live-Dead project.

PCR is a highly sensitive and quantitative way of detecting bacteria. However, unlike culture, it does not distinguish between living and dead bacteria. This is because it is the organism's DNA that is detected and DNA is a stable molecule that may persist for many weeks after the cell is dead. This can lead to misleading results; for example when using PCR for detection of *Legionella* in a hot water system, a PCR test may well show positive results even though the system has been adequately sanitized because dead cells are still present.

We are investigating two approaches to distinguishing live and dead bacteria in PCR tests:

- **Phenanthridine monoazide inactivation.** Phenanthridine monoazides (PM) such as ethidium monoazide are dyes that bind to DNA and inactivate it in the presence of light. Such dyes are excluded from living cells. In principal, sample treatment with PM and light should eliminate PCR signals from dead cells and free DNA, leaving only the signal from living cells.
- **RNA PCR.** Unlike DNA, RNA has a short half life and quickly disappears from dead cells. RNA can be detected by PCR if it is first converted to DNA using the enzyme reverse transcriptase. We are investigating RNA-based detection systems to distinguish live from dead cells.