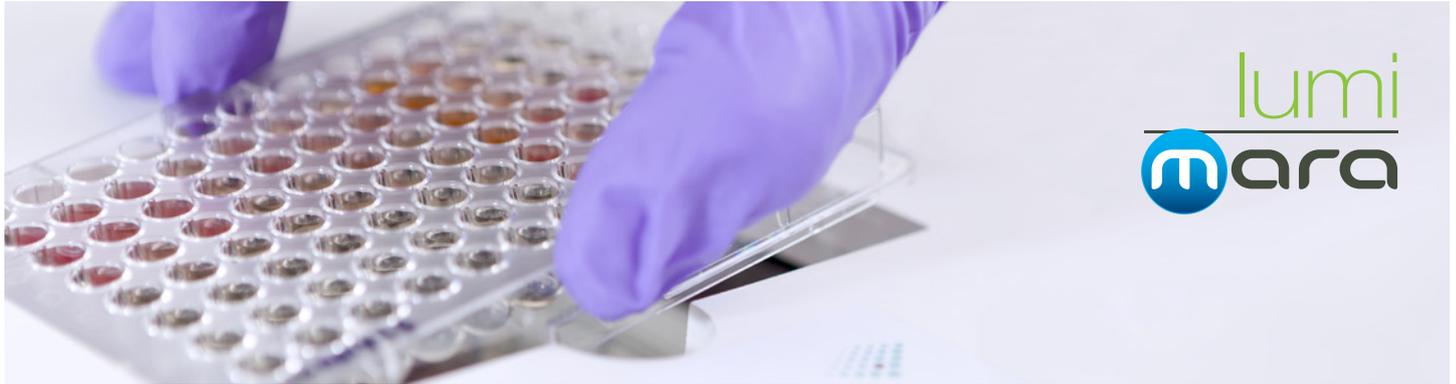




A Guide to the LumiMARA Microbial Ecotoxicity Test



NCIMB's LumiMARA ecotoxicity test is a multispecies microbial bioassay. It uses reduction in light output by 11 luminescent microbial strains to give a measure of toxicity.

It offers a more diverse alternative to ecotoxicity testing than using a single luminescent bacterial strain, whilst retaining the benefits of a microbial test.

LumiMARA can be used to test waste materials and effluents, chemicals, or soil and sediment samples.

Introduction

Go to:

Introduction

Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits or a multispecies test

Track record

Additional services

Ecotoxicity testing

Ecotoxicity testing gives an insight into the impact that chemicals or effluents could have on the environment. It can also be a useful tool for assessing sites or areas for the presence of toxic compounds. Testing involves exposing living organisms to test substances and assessing the effect. Living organisms commonly used for testing can include bacteria, algae, crustacea, and fish.

Microbes

Microbes have a number of benefits for ecotoxicity testing compared to higher order organisms.

1. **Convenient format** – bacteria are small and easy to handle in large numbers, so for example, testing can be carried out in a test tube or microtiter plate.
2. **Fast:** Bacteria grow and respond quickly to the presence of toxins, so test results can be obtained quickly.
3. **No ethical issues.** Testing with microorganisms has none of the ethical issues associated with testing using higher order organisms such as fish.

Go to:

Introduction

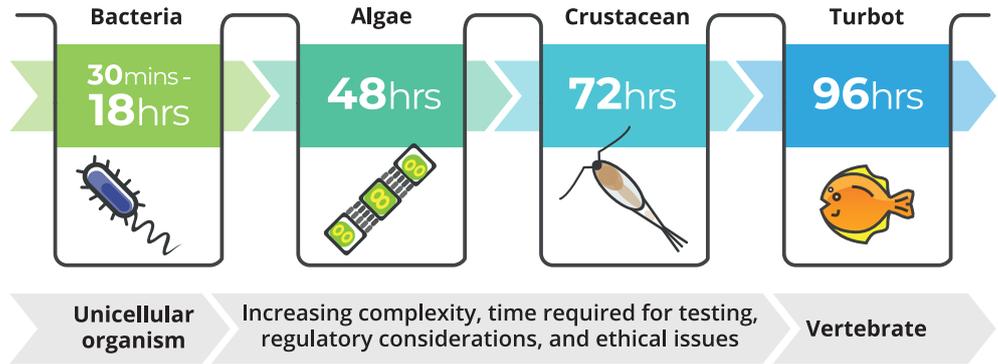
Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits or a multispecies test

Track record

Additional services



Microorganisms as bioassays

Microorganisms

Go to:

Introduction

Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits or a multispecies test

Track record

Additional services

The use of microorganisms in ecotoxicity testing is well established. A well-known and widely used example is the microbial bioassay *Aliivibrio fischeri* (previously known as *Vibrio fischeri*).

A. fischeri is a species of naturally luminescent marine bacteria. Reduction in light output following exposure to test substances provides a very fast measure of toxicity. Methods for ecotoxicity testing using a strain of the species *A. fischeri* are described in a three-part ISO standard.

However, there are several different strains of *A. fischeri*, for example there are 11 listed in the NCIMB culture collection catalogue, and there are many other different species of luminescent bacteria. They are most commonly associated with marine environments, but some also live in freshwater or soil.



[Go to:](#)

[Introduction](#)

[Microorganisms as bioassays](#)

[LumiMARA Ecotoxicity test](#)

[Benefits or a multispecies test](#)

[Track record](#)

[Additional services](#)

ISO standard: Luminescent bacteria test

ISO 11348-1:2007	Water quality - Determination of the inhibitory effect of water samples on the light emission of <i>Vibrio fischeri</i> (Luminescent bacteria test) - Part 1: Method using freshly prepared bacteria
ISO 11348-2:2007	Water quality - Determination of the inhibitory effect of water samples on the light emission of <i>Vibrio fischeri</i> (Luminescent bacteria test) - Part 2: Method using liquid-dried bacteria
ISO 11348-3:2007	Water quality - Determination of the inhibitory effect of water samples on the light emission of <i>Vibrio fischeri</i> (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria

LumiMARA

Ecotoxicity test

Go to:

Introduction

Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits or a multispecies test

Track record

Additional services

LumiMARA is an ecotoxicity test that builds on the benefits of testing using a single species of bacteria by including 11 different bioluminescent strains.

One of them is a strain of *A. fischeri* that is equivalent* to the strain referenced in the ISO standards. Although they are all bioluminescent bacteria, they exhibit genetic diversity and were isolated from different environments. For example, nine are marine strains and two are freshwater strains.

Using LumiMARA instead of testing with a single species of *A. fischeri*, gives all the benefits of microbial testing, with greater diversity, which in turn can give greater sensitivity.



*Equivalency in bacteria

- All culture collections give a unique identifier to each of the strains in their collections, and there is some overlap in the strains that major collections hold.
- Overlaps arise when scientists deposit strains in more than one collection, or collections share strains. The same strain will be given a different identifier in different collections.
- It is generally accepted that when a major culture collection states that e.g. *Pseudomonas aeruginosa* NCIMB 8295 = NCTC 10332 = DSM 50071 = CECT 110, that these cultures will be indistinguishable from one another.

Go to:

Introduction

Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits or a multispecies test

Track record

Additional services



GENETICALLY DIVERSE

More genetically diverse than single species testing



MARINE AND FRESHWATER STRAINS

Representative of different environments



INCLUDES TWO ALIIVIBRIO FISCHERI STRAINS

One of which is equivalent to NRRL B-11177 referenced in ISO 11348-3

LumiMARA

Go to:

[Introduction](#)

[Microorganisms as bioassays](#)

[LumiMARA Ecotoxicity test](#)

[Benefits or a multispecies test](#)

[Track record](#)

[Additional services](#)

Benefits of a multispecies test

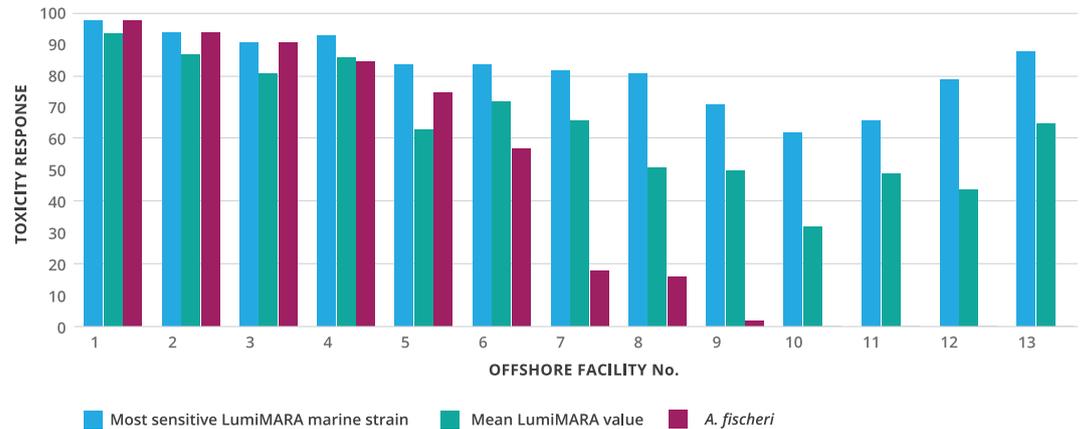
Data from previous work shows the benefit of testing using the multi species LumiMARA test in comparison to testing using the single species of *A. fischeri* specified in the ISO standard.

When the eleven different species within the test are exposed to the substance to be tested, they display a range of different responses. The results obtained can be analysed in relation to the most sensitive strain within the test, or the mean result of all strains.

- The chart (on page 9) shows the toxicity response of the *A. fischeri* strain, compared to the response of the most sensitive strain within the LumiMARA test and the overall lumiMARA result.
- The data was obtained from testing of produced water from North Sea oil and gas production platforms.
- It shows that in some cases testing with *A. fischeri* alone shows little or no toxic effect, when both the most sensitive strain and the mean result show medium to high levels of toxicity.

Go to:

- Introduction
- Microorganisms as bioassays
- LumiMARA Ecotoxicity test
- Benefits of a multispecies test
- Track record
- Additional services



The data above has been plotted in order of declining *A. fischeri* response. For some effluents tested, *A. fischeri*, the mean LumiMARA and most sensitive LumiMARA strain all show similar toxic responses, but in other cases e.g. facilities no 10-13, testing with *A. fischeri* alone shows no toxic response while LumiMARA indicates medium to high levels of toxicity.

LumiMARA

Track record

Go to:

[Introduction](#)

[Microorganisms as bioassays](#)

[LumiMARA Ecotoxicity test](#)

[Benefits or a multispecies test](#)

[Track record](#)

[Additional services](#)

- LumiMARA has been used extensively for testing of produced water/effluent in the oil and gas sector.
- It is recommended by the UK's Department of Business, Energy and Industrial Strategy (BEIS), as part of their preferred approach to the management of produced water from offshore installations. The recommendation was made following offshore trials.
- The tests have also been used for the assessment of oil and gas production platform decommissioning waste, for toxicity assessment of chemicals and in research projects as well as for testing a variety of other effluents.

	<i>A. fischeri</i>	LumiMARA
Includes <i>A. fischeri</i> NRRL B-11177 or an equivalent strain	✓	✓
Toxicity assessed by reduction in light output	✓	✓
Number of marine strains included	1	9
Number of freshwater strains	0	2
Total number of strains	1	11

LumiMARA

The multispecies microbial ecotoxicity test for more informed decision making.

Go to:

Introduction

Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits of a multispecies test

Track record

Additional services

Microorganisms are an extremely diverse category of living things and different species can have very different responses to effluents and chemicals. Microbial ecotoxicity tests can be used for screening prior to higher order tests, but understanding toxicity of substances to microorganisms is also important in itself, as microbial life is essential for the health of the whole ecosystem.

With LumiMARA it is easy to incorporate more species into testing, providing more data, with which to make a more detailed and robust assessment.

Please contact enquiries@ncimb.com for details.



Additional services

NCIMB offers a range of additional testing services

Go to:

Introduction

Microorganisms as bioassays

LumiMARA Ecotoxicity test

Benefits or a multispecies test

Track record

Additional services

Effluent testing suite

We offer a suite of commonly requested chemical analyses and tests for effluents and oilfield produced waters including:

- Chemical oxygen demand (COD)
- Biological oxygen demand (BOD)
- PH
- Total suspended solids
- Total bacteria
- Sulphide/sulphate
- Chloride/chlorine

Microbial community analysis

At NCIMB we use the most accurate sequencing technology to help customers understand and monitor whole microbial communities from a range of samples, including industrial effluent and environmental samples. We undertake 16S metagenomic sequencing to help understand the make up of microbial communities, and analyse changes over time.

qPCR

Some groups of microbes, eg sulphate reducing bacteria, can cause foul odours in waste waters. We can develop customised assays for organisms/ functional genes of interest or undertake commonly requested tests.

Contact us for a quote and more information on how to send and package samples

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