

## **TECHNOLOGY OVERVIEW – ToxScreen<sup>3</sup> Test**

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### **Why is it important to determine chemical contamination in water?**

The quality of potable water is one of the most important elements for human health. Intense development of the chemical industry, and the use of pesticides in agriculture can result in contamination of natural water resources. Moreover, chlorinating of water (used to inhibit bacterial contamination) may lead to the generation of complex mixtures of toxic and genotoxic chlorinated hydrocarbons in drinking water. In the absence of a rapid, sensitive, general, and cost-effective test for such a diverse group of contaminants, however, most water supplies are not monitored on a frequent routine basis.

### **The principle of the ToxScreen test**

The use of intact luminous bacteria for toxicity assessment has some clear advantages that have been scientifically validated (Bulich and Isenberg, 1981; Kaiser, 1998; Ulitzur et al, 2002): luminous bacteria are self-maintained luminescent units that, under proper conditions, emit high and steady levels of luminescence (490nm). The light of several hundred cells may be determined with a simple luminometer.

Chemo-physical and biological toxicants that affect cell respiration, electron transport systems, ATP generation, the rate of protein or lipid synthesis, alter the level of luminescence. Similarly, agents that affect the cell's integrity and especially membrane function, have a strong effect on in vivo luminescence. Hence, toxicants of different characteristics such as, pesticides, herbicides, chlorinated hydrocarbons, heavy metals etc, exert a dramatic and measurable effect on the bacterial luminescence system. By comparing the luminescence level obtained in a suspected toxic sample with that obtained in a clean water-control sample after a short period of incubation, one can detect very low concentrations of a broad range of toxicants.

The ToxScreen test is based on using luminous bacteria as very sensitive and accurate biosensors. The operation principle is simple – changes in the level of bio-luminescence indicate potential toxicity. The test kit includes Assay buffers, freeze-dried luminous bacteria, positive control solutions, and plastic tubes. A luminometer is required to read the

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results. The testing procedure is simple as well: 3 steps that can be performed under field conditions in ambient temperature (18-35°C). Only 1mL of sample is necessary to run the assay, making collection, storage, and disposal of sample material easy and non-expensive. Low cost per test encourage frequent testing for rapid response to changing conditions in water quality. The tests can be performed **either in the lab or on site** and can be used by water companies, health and environmental supervising authorities, municipal water system authorities, hospitals, military units, private well owners, lake, stream and river monitoring agencies etc.

### **Why is the CheckLight ToxScreen Test more sensitive than other short-term bioluminescence tests?**

The higher sensitivity of the ToxScreen test to diverse groups of toxicants with comparison to other bioluminescence based tests is due to the selection of a highly sensitive variant of *Pothobacterium leiognathi*, an improvement of its lyophilization procedure and the selection of special assay conditions. This unique combination resulted in 10 to 100 folds increase in sensitivity for most of the important toxic agents (see Table 1 for a few examples). Two assay buffers were developed, one which favors the detection of cationic heavy metals and metalloids (Pro-Metal Buffer), and the other favors the detection of organic contaminants (Pro-Organic Buffer). These buffers were developed to enhance the sensitivity of the test to a wide range of toxic agents with different modes of action. When tested concurrently, the buffers are generally able to discriminate between the presence of organic and metal agents at sub mg/L concentrations.

It is important to note, however, that anionic metals (chromium) will be more active in the Pro-Organic Buffer; while cationic organic toxicants (diquat, paraquat) will be more active in the Pro-Metal Buffer. The added advantage of including strong buffers in the assay system lies in minimization of the artefactual toxic effect of extreme pH occasionally encountered when using other bioluminescence-based assays having only NaCl solution as the primary exposure medium.

### **What is unique about the CheckLight ToxScreen<sup>3</sup> Test and how is it different from the ToxScreen-I and -II Test?**

The modified **ToxScreen<sup>3</sup>** test (introduced in late 2007) offers increased sensitivity for many

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chemical contaminants, rapid results (reaction time shortened from 60 minutes to **15 minutes**), added flexibility due to reagent work week-long utilization, and wider temperature tolerance. The test is based on the effect of toxic agents on the development of luminescence in the natural marine bacterium *Photobacterium leiognathi* (strain SB) that is more temperature tolerant than the *Photobacterium leiognathi* variant TANI-1 used in the ToxScreen-I version.

**Sensitivity** - the ToxScreen<sup>3</sup> exhibits improved sensitivity to a wide range of toxic agents, some of which are known as potential terrorism hazards (arsenic, cyanide) and others (2,4-D, 2,4,5-T, and chromium - see Table 1). Unlike *Vibrio fischeri* or even the TANI-1 variant, the bacterial strain used is very tolerant to a wide range of temperatures (18°-35°C), though the optimal temperature for rapid response is 28°C.

**On site real time results** - one of the key features of the new test is its rapid response. Optimal results can be obtained on site within **15 minutes**. The kinetics of response largely depends on the nature of toxic chemical and its concentration. Hence, in many cases a detectable effect on luminescence will be observed within a few minutes of exposure. When real time results are crucial, one could record luminescence after 5 and 10 minutes.

**Cost efficiency** - an important advantage of the new test is the extended usability of the suspended reagent; unlike the ToxScreen-I test version, the hydrated luminous bacteria are transferred into Storage Buffer and stored at 2°-4°C. Around 100 single tests may

be drawn from this suspension for up to **5 days**. During the 5 days storage, light output diminishes but sensitivity and reliability of results remains stable. The bacterial suspension is added to the tested water to which a buffer containing certain salts and metabolites is added by the user. In the absence of toxicants the in vivo luminescence remains quite stable at 26°C (+/- 3°C). For most of the toxic agents tested, the new assay was markedly more sensitive than comparative bacterial bioluminescence toxicity data reported in the literature with other bioluminescence-based short-term tests. As with ToxScreen-II, the robustness of the SB variant enables on-site testing with low cost luminometers. In addition, the stability of the freeze-dried bacterial reagent preparation at ambient temperatures precludes the need for refrigeration or freezing contributes to further reducing overall operational costs.

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## Conclusions

ToxScreen enables water utilities and supervising organizations to better protect public health. ToxScreen provides an **early warning** of dangerous water contaminations due to spills, accidents, sabotage or terrorism. ToxScreen enables rapid screening for changes in water quality in surface and ground water sources, as well as water distribution networks.

## References

- Bulich AA, Isenberg DL. 1981. Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity. ISA Transactions, 20: 29-33.
- Kaiser KL. 1998. Correlations of Vibrio fischeri bacteria test data with bioassay data for other organisms. Env. Health Perspect. Apr;106 Suppl 2:583-91.
- Ulitzur S. Lahav T. Ulitzur N. 2002. A Novel and Sensitive Test for Rapid Determination of Water Toxicity. Environmental Toxicology Journal 17:291-296.

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