

Technical Note No. 5:

## **COLIFAST FAST**

**Rapid test to detect spoilage bacteria in fresh fish.**

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Fax: + 47 67 10 05 20e-mail: [post@colifast.no](mailto:post@colifast.no) <http://www.colifast.no>**COLIFAST FAST: Rapid test to detect spoilage bacteria in fresh fish.****Introduction:**

*Shewanella putrefaciens* has been identified as the most important spoilage bacterium of temperate and tropical marine fish stored aerobically in ice (Huss, 1995; Gram 2003). Other bacteria, which may be of importance during spoilage of temperate and tropical marine fish at ambient temperature are *Vibrio* spp. (Huss, 1995; Gram, 2003). These bacteria are characterised as sulphide producing bacteria (SPB), which during growth and metabolism produce the rotten sulphur off-flavour associated with fish spoilage (Gram and Huus, 1996). The foul-smelling of these volatile sulphur compounds can be detected at ppb levels (Huss, 1995). Minimal quantities of these compounds will consequently have a considerable effect on quality, and the number of SPB in fish is well correlated to the remaining shelf-life (Jørgensen et al., 1988, Dalgaard et al., 2002, Gram et al. 2002).

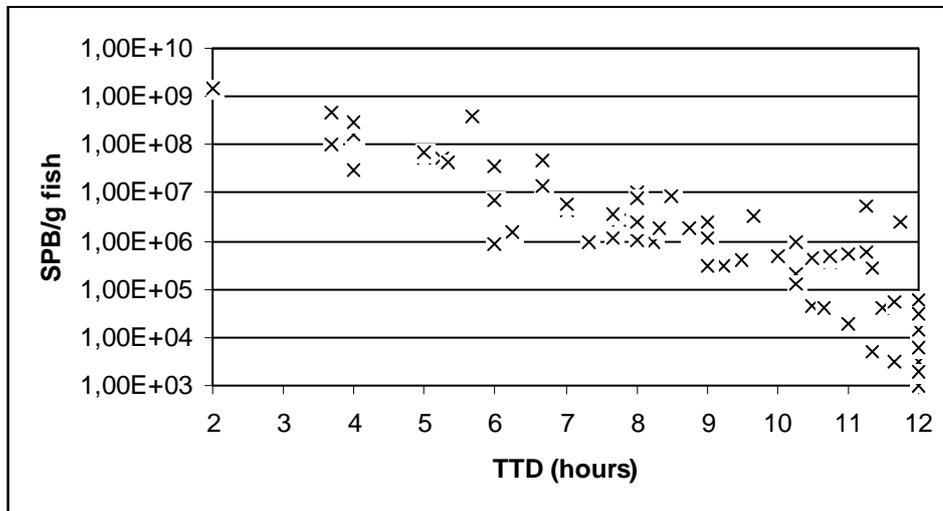
Based on the need for a rapid test to quantify the SPB in fresh marine fish Colifast AS together with Norwegian Institute for Fisheries and Aquaculture (Fiskeriforskning) embarked on a 3 year R & D programme funded by the Norwegian Research Council and MABIT (Marine Biotechnology in Tromsø) (Skjerdal et al., 2003). The project was followed by an external evaluation at the Norwegian Food Control Authority in Borg, and at commercial sites like fish traders and fishery industry (Lorentzen and Tryland, 2003). As a result of the project a test designated FAST (Fast Sulphide producing bacteria Test) was developed, which has international patents and patents pending.

**Methodology:**

Quantification of SPB is currently performed by the traditional spread plate method, where colonies formed on a customised medium are counted (Gram et al., 1987). During growth of the SPB sulphur compounds are converted to sulphide, which precipitates as FeS, in turn producing black colonies on the solid medium. In order to maximise counts, incubation for three days is required. Moreover, laboratory facilities are needed together with skilled personnel, the detection level of the spread plate method is high (50-100 CFU/g), and early warning in cases with high levels of SPB is impossible.

The FAST test consists of a modified growth medium for SPB as that described by Gram et al. 1987. The FAST test is designed to permit early warning of high levels of SPB, to allow untrained personnel to perform the analysis, and to minimise the need for laboratory facilities during the analysis. Approximately 1cm<sup>3</sup> fish sample is placed in a vial containing the FAST medium, and incubated at 30°C. During the FAST test a visible colour change from clear yellow to black appears in the liquefied FAST medium, due to precipitation of FeS. This visual observation is a simplification of the FAST method as described by (Skjerdal et al., 2003), where colour change was measured by fluorescence. The time to detect the colour change (TTD) is correlated to the number of SPB obtained and consistent with the method of

Gram et al. 1987. In Figure 1, the correlation between number of SPB and TTD is given. These samples were taken from fresh fish distributors and stores in June-August 2003, and consisted of indigenous SPB.



**Figure 1.** The correlation between TTD and number of SPB/g fish. A log transformation of the number of SPB versus TTD gives a Pearson's correlation coefficient ( $r^2$ ) of -0.90 (n= 78).

The colour change in the FAST medium is observed visually as shown in Figure 2. The series of vials shows the progression of FeS precipitation as blackening of the media. Levels of SPB are then estimated from the time that it takes to make the colour change in the FAST medium (Table 1).



**Figure 2.** Visual reading of the colour change in the FAST medium.

**Table 1.** Interpretation of TTD and levels of SPB.

Hours to colour change Yellow → Black	SPB/g	Quality Guideline*
3-6	> 5 000 000	Poor
7-10	500 000 - 5 000 000	Marginal
11-12	1000 - 500 000	Good
> 12	< 1000	Very good

\* Values may vary slightly depending on application.

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**Application areas:**

Potential application areas of FAST include all steps in production and distribution of fresh fish:

- QC testing in receiving areas to insure that chilled storage has not been interrupted.
- Routine QC testing of suppliers, both old and new.
- Sorting of raw materials for different uses.
- Determination of remaining shelf life of raw materials in question.
- Routine testing of hygienic status of the production area.
- Use FAST in internal quality control systems.
- Use FAST to gain a competitive advantage. Proactive suppliers who are ahead of regulations give confidence to customers.

**References:**

Dalgaard, P., Buch, P. and Silberg, S. 2002. Seafood Spoilage Predictor – development and distribution of product specific application software. *International Journal of Food Microbiology* 73: 343-349.

Gram, L. 2003. Homepage and lecture notes of Lone Gram, Department of Seafood Research, Danish Institute for Fisheries Research, <http://www.dfu.min.dk/micro/lg.htm>, revised June 2003.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., and Givskov, M. 2002. Food spoilage – interactions between food spoilage bacteria. *International Journal of food Microbiology* 78: 79-99.

Gram, L and Huus, H.H.1996. Microbial spoilage of fish and fish products. *International journal of food microbiology* 33: 121-137.

Gram, L., Trolle, G., and Huss, H.H. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. *International Journal of Food Microbiology* 4: 65-72

Huss, H.H. (ed.) 1995. *Quality and Quality Changes in Fresh Fish*. FAO Fish. Techn. Pap. No. 348. 195 p

Jørgensen, B.R., Gibson, D.M., and Huss, H.H. 1988. Microbiological quality and shelf life prediction of chilled fish. *International Journal of Food Microbiology* 6: 295-307.

Lorentzen, G. and Tryland, I. 2003. Utvikling og ferdigstillelse av en hurtigmetode for dokumentasjon av råvare- og produktkvalitet i sjømatindustrien (in Norwegian). Report 7/2003, 23 p. (ISBN82-7251-517-2), Fiskeriforskning.

Skjerdal O.T., Lorentzen G., Tryland I., and Berg, J.D. 2003. New method for rapid and sensitive quantification of sulphide producing bacteria in seafood (manuscript submitted to *Journal of Food Science*).