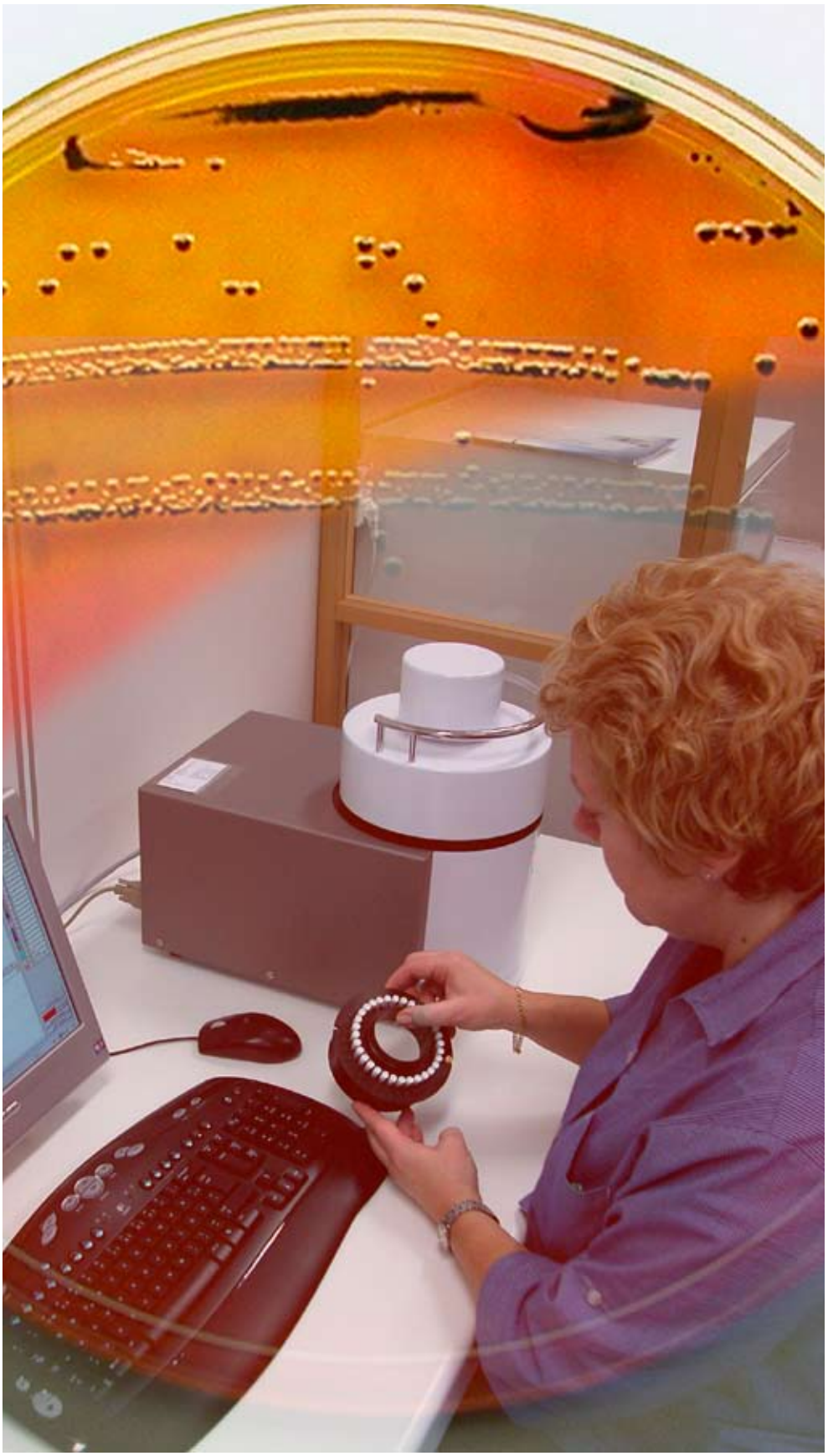
Salmonella detection with RealTime PCR



Nofima Ingrediens has received accreditation of a new method for Salmonella detection in feed, feed ingredients, foods and environmental samples. The method, which is based on Real-Time PCR (Polymerase Chain Reaction), provides negative or presumptive positive results in only 24 hours. This is the fastest available method for Salmonella detection today. Presumptive positive samples are confirmed by a conventional method.

NFSA requirements

The Norwegian Food Safety Authority (NFSA) is the competent authority for food and animal feed in Norway. NFSA has the legal power to control, supervise and approve production plants and products. Product approval is based on accredited analyses performed with internationally recognised methods or with methods demonstrated to give results equivalent to that of specified reference methods.

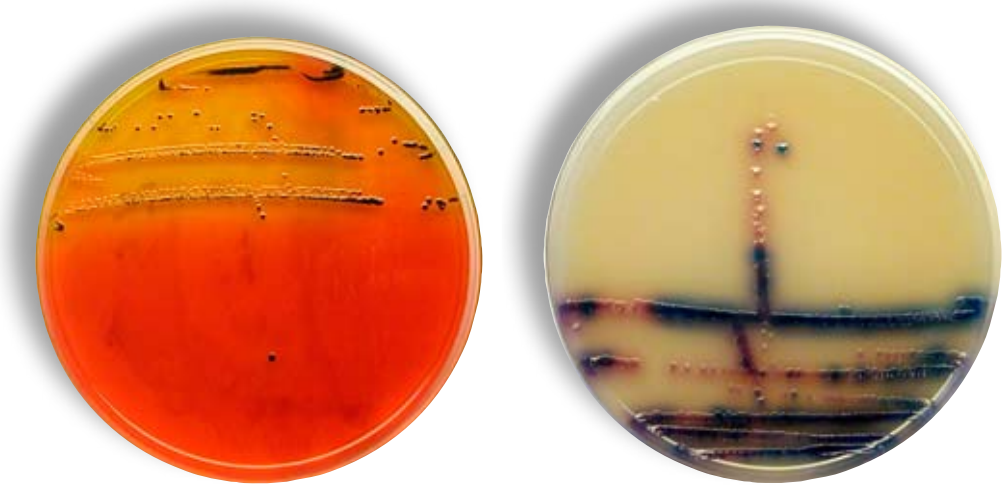
Reference methods

In the Nordic countries, the conventional culture method issued by the Nordic Committee on Food Analysis, NCFA 71, is recognised as a reference method for Salmonella analysis. NCFA 71 is similar and equivalent to the international reference method ISO 6571. Any alternative methods have to be validated against a recognised reference method.

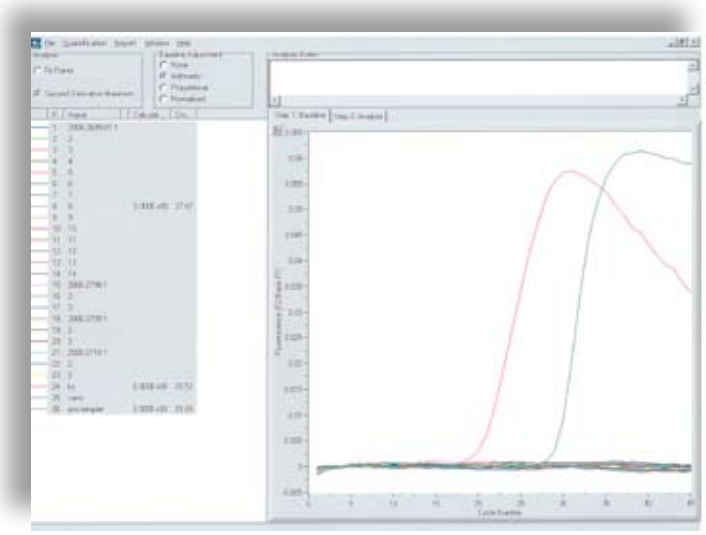
Principles of the combined RealTime PCR/ NCFA 71 method

Real-Time PCR is a process for mass copying and real-time detection of DNA segments. Known "target DNA" segments are copied by means of a reagent mix (primers, probes, enzymes, etc.). The process is controlled by a temperature cycle that is repeated 45 times, theoretically resulting in up to 35,000,000,000,000 (2^{45}) copies of the target DNA. The PCR machine used by Fiskeriforskning takes 50 seconds per cycle and a PCR run is completed in less than one hour.

Before PCR analysis, the samples are subject to pre-enrichment (20 h), sub-cultivation (3 h) and DNA extraction (0.5 h). The total duration of an analysis is therefore about 24 hours. PCR negative analyses are terminated, while PCR positive analyses are completed with NCFA 71, involving selective enrichment, isolation and biochemical/ serological confirmation of Salmonella. Available Salmonella isolates are a prerequisite for further verification/serotyping, and may also be required by NCFA as a basis for epidemiological investigations.



A critical step in NCFA 71 is the isolation of pure Salmonella colonies from selective-diagnostic agar media. Salmonella forms characteristic magenta-coloured colonies on OSCM agar (right) and black colonies on XLD agar (far right).



The screen shows that "target DNA" from Salmonella is detected in 2 of 26 samples. One of them is a positive template, which is analysed parallell with the sample DNA extracts to control the process. In addition, internal control DNA is included in every test capillary in order to reveal product inhibition, i.e. inhibition of the PCR process due to sample constituents. The internal control DNA is detected at a separate wave length (not shown).

Laboratory routines

Salmonella analyses are normally started the same day the samples are received. To ensure speed and reliability, the laboratory is regularly manned on weekends and holidays. Five analysts are approved for independent Salmonella analysis. The same day as the analyses are completed, an analysis report is sent to the customer per fax. Reports are also sent per post.

LightCycler 1.5 instrument (Roche Diagnostics, GmbH, Germany) uses air instead of a conventional thermoblock for heating and cooling. This contributes to a faster and more uniform temperature distribution in the reaction capillaries. The sample carousel has a capacity to hold up to 32 samples.

Method validations

Organisation	# samples	Relative sensitivity (%)	Relative specificity (%)	Detection limit # per 25 g
NorVal [1)]	840	100	99	1
AOAC [2)]	440	100	100	1 - 10
Fiskeriforskning [3)]	60	100	100	1 - 10

1) NorVal Certificate for Roche Diagnostics LightCycler Foodproof Salmonella Detection Kit in combination with ShortPrep Foodproof I Kit (Ref.no.: 2005-30-5408-00052).
2) AOAC Certificate of Performance Tested Status. Roche Diagnostics LightCycler Foodproof Salmonella Detection Kit for Salmonella sp. in combination with ShortPrep Foodproof I Kit (Certificate no. 120301).
3) Validation of alternative methods for Salmonella detection (Fiskeriforskning Report K-306)

Accreditation

Fiskeriforskning is accredited (TEST 045) by Norwegian Accreditation (NA) according to NS-EN ISO/IEC 17025 for the areas chemistry and microbiology. The PCR method for Salmonella detection was granted accreditation by NA in October 2006. Accreditation is an official recognition of the technical competence of a body to perform specific tasks.

Read more

http://www.nofima.no

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– Creating value together

Nofima is a business oriented research group working in research and development for the aquaculture, fisheries and food industry in Norway.
Nofima was established on January 1, 2008, and has about 470 employees.
The group's total turnover in 2008 was about NOK 470 million.

The Nofima Group is divided into the following four business divisions:

Nofima Marin (marine)
Nofima Marin engages in research, development, innovation and knowledge dissemination for the national and international fisheries and aquaculture industries. Our core areas are breeding and genetics, feeds and nutrition, fish health, efficient and sustainable production, process and product development of seafood and marine bioprospecting.

Nofima Mat (food)
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Nofima Ingrediens (ingredients)
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Nofima Marked (market)
Nofima Marked is our analytical business division, which provides economic analyses, foresight analyses, consumer research, market analysis and strategic consultancy. We can also offer consultancy in information logistics and traceability.

Our offices
The group's head office is located in Tromsø in Northern Norway, while the research divisions are located in six places throughout Norway: Ås, Stavanger, Bergen, Sunndalsøra, Averøy and Tromsø.