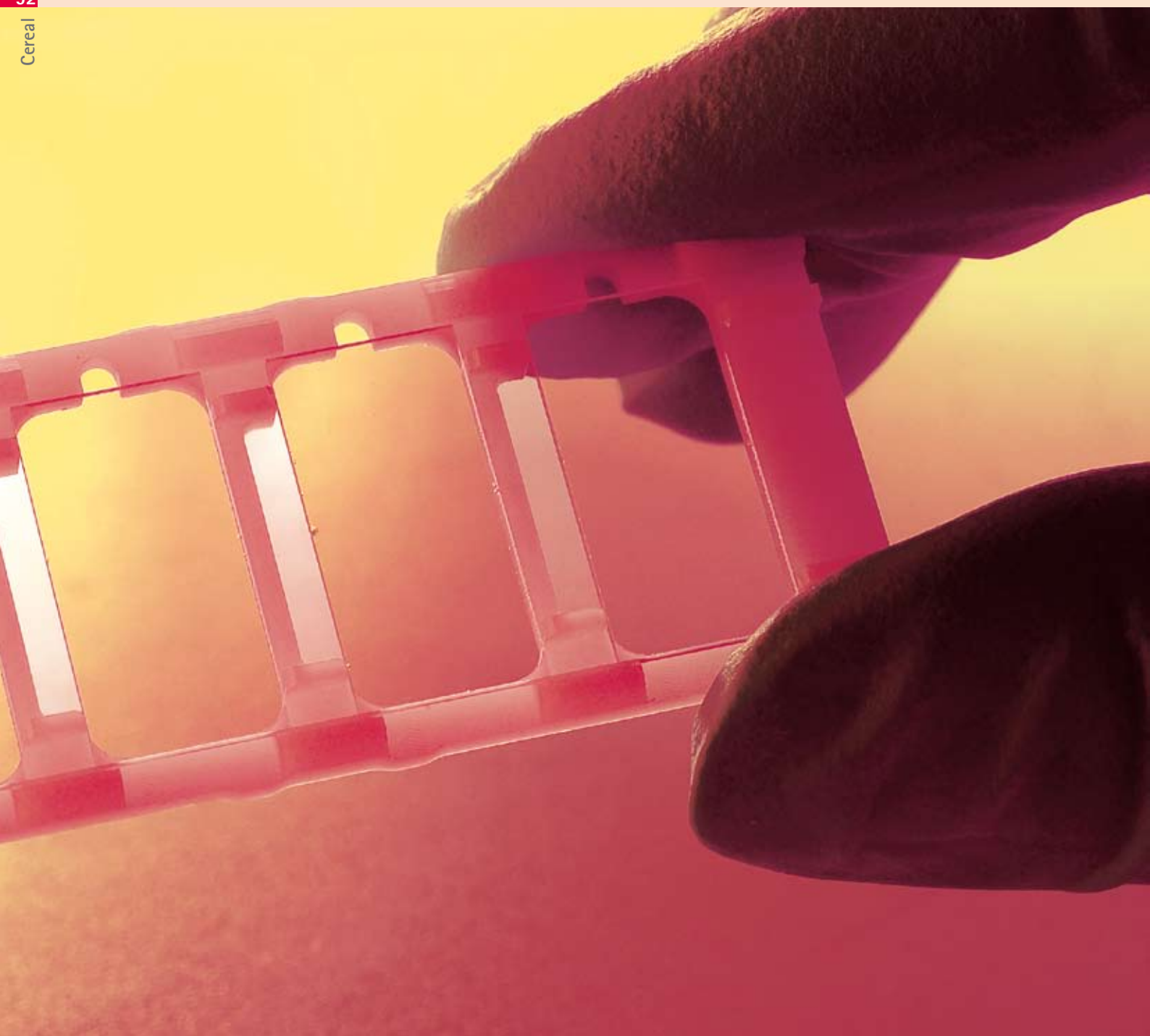
Highly accurate rapid diagnostic test developed for cereal

The **mycotoxin** detective



Molds leave residues in foods which can be harmful to health. A Bayer interdisciplinary team comprising physicists, biochemists, chemists and information technologists from Bayer CropScience and Bayer Technology Services is developing a measuring device which can accurately determine the level of the five most common mycotoxins in cereals within only 20 minutes of arrival at the cereal depot.

Toxin diagnosis: using glass biochips (left), scientists can determine within a few minutes how heavily a batch of cereal is contaminated with mycotoxins – before it is mixed with other batches in storage silos (above right).

The harmful effects of mycotoxins were known before there was even a word for these poisons: ergotism, a disease which occurs after eating ergot, was described in the Bible, for example. Today, mycotoxin is the collective name for various metabolic products of molds, for example Fusarium. Some mycotoxins can cause acute symptoms of poisoning, nerve damage and immune disorders or can even be carcinogenic to humans and animals.

Cereals can also become contaminated with Fusarium toxins, either in the field or during storage. The European Union therefore issued maximum limits for certain toxins in unprocessed cereals on July 1, 2006, including the Fusarium toxins deoxynivalenol (DON) and zearalenone (ZEA).

At present, the contamination of cereal is assessed mainly by appearance. "If cereals are contaminated with Fusarium molds, the grains turn a reddish color," explains fungal expert Dr. Friedrich Kerz-Möhlendick of Bayer CropScience. "The mycotoxin content is usually deduced from this red color. Although there are various detection methods for mycotoxins on the open market and there are even rapid tests, many users regard them as imprecise, as the results they give are qualitative rather than quantitative."

For precise determination of the mycotoxin content, therefore, samples have to be taken from cereal deliveries and subjected to elaborate tests in specialist laboratories. The results of these tests take several days to return. By that time, individual deliveries of cereals can no longer be identified in the storage units, which hold up to 4,000 tons. Cereals which would meet the highest requirements may now be lying underneath contaminated cereals.

Cereal tester takes minutes instead of days

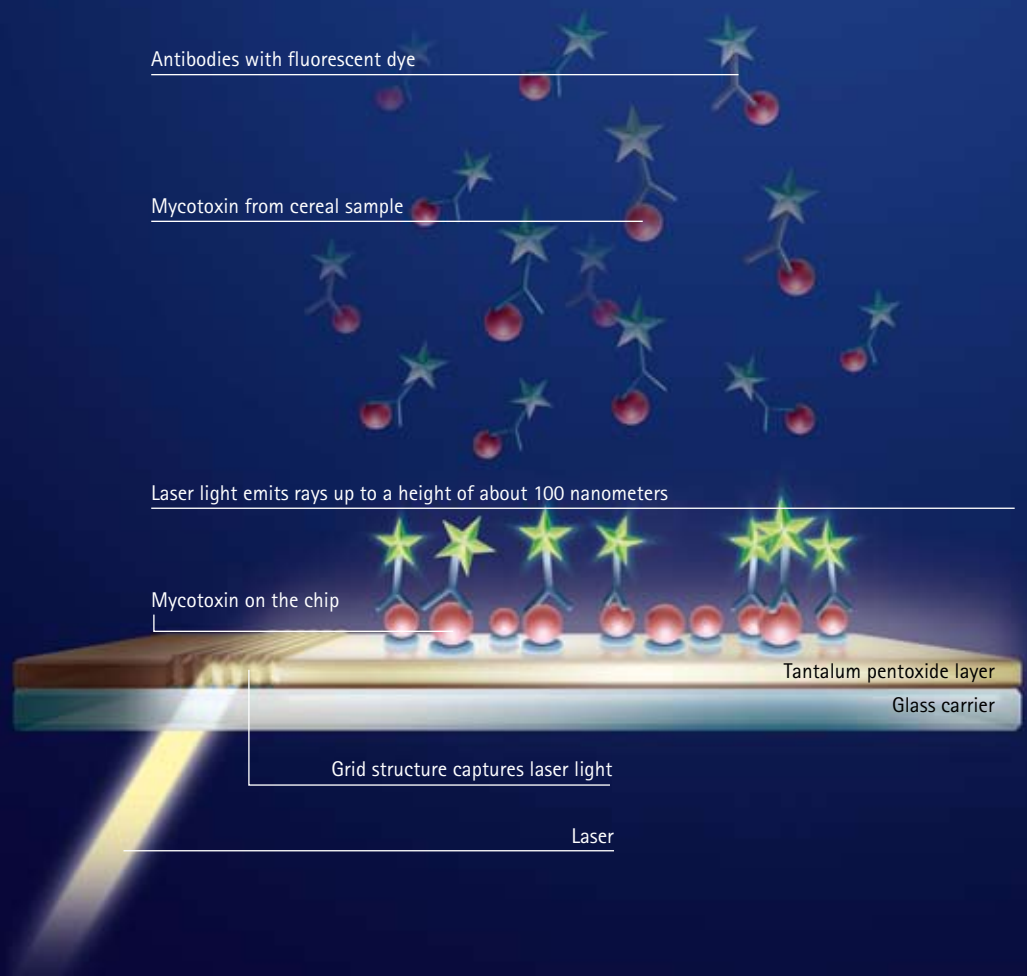
This is where the diagnostic test developed by Bayer comes in. "The aim is to determine the precise mycotoxin content of the cereal before it is placed in the silo for storage," explains project leader Dr. Jens Burmeister of Bayer Technology Services. "We are developing a test device which can deliver precise results on the spot in just 20 minutes." The entire diagnosis process takes place on a tiny centimeter-square biochip which is read out precisely with the aid of a laser. The technique used here - the planar waveguide technology developed by Zeptosens - has its origins in medical diagnostics, and is impressive in terms of both speed and accuracy (see *research* 16).

The basic principle is simple: the mycotoxins are isolated from the cereal grain using methanol. This cereal extract is mixed with antibodies to the mycotoxin being tested for, which are labeled with a fluorescent substance. A tiny

Image analysis: Viktoria Bazilyanska uses the prototype reader to evaluate the mycotoxin assay taken with the biochip. Each of the blue dots on the screen indicates the light intensity of a measurement on the chip.

No competition for illumination

To determine the mycotoxin content, the mycotoxins are extracted from the cereal, mixed with fluorescence antibodies and applied to the biochip. The mycotoxins on the biochip compete with the mycotoxins in the sample to bind to the antibodies: the more mycotoxins there are in the sample, the fewer antibodies bind to the chip. Only these antibodies then respond when the chip is evaluated with laser light – the tantalum pentoxide layer makes sure that the laser light only emits rays up to a low height.



drop of the mix is placed on the biochip. Mycotoxin molecules, termed scavenger molecules, are also fixed on the chip at defined spots. A competition situation now occurs: the marked antibodies can either bind to the scavenger molecules on the chip or to the free mycotoxin in the cereal extract. The more mycotoxin there is in the extract – i.e. the more contaminated the cereal – the fewer antibodies bind to the mycotoxin spots on the chip. The researchers call this an indirect competitive immunoassay.

Antibodies and lasers reveal mycotoxin concentrations

When the chip is then illuminated with laser light, the fluorescing antibodies send back a specific light signal which is picked up and evaluated by a special camera. Reference spots on the chip allow the fluorescence signals to be quantified. The more mycotoxin there is in the cereal extract, the weaker the light given off by the chip.

Putting this reaction into practice requires high technology in the tiniest of spaces: manufacturing a biochip measuring only one square centimeter requires utmost precision. The read-out is also complicated: how can the fluorescence signals from the chip be distinguished from those from the cereal extract, without having to wash out the extract in several error-prone stages? This is where planar waveguide technology comes in - it only measures the fluorescence in an extremely thin

Biochip manufacture: a pipetting robot (right) applies precisely dosed drops of various mycotoxins to a chip in a fully automatic procedure. The mycotoxins are attached to the surface of the chip by another protein.

layer directly over the chip. The chip has a grid structure which is able to capture laser light. For the read-out, a laser beam is guided on to the chip and the grid structure guides the laser light into a transparent, highly refractive layer which transports the light as if it were in a glass fiber cable. The key point is that the light intensity decreases exponentially the further away it is from the surface. Up to a height of about 100 nanometers above the chip, there is a strong light signal, yet above this there is hardly any signal at all.

It is precisely within this approximately 100 nanometer layer that the fluorescence-labeled antibodies bind to the spots on the chip, thereby ensuring that only the labeled antibodies which have bound to the scavenger molecules on the chip are able to send out a fluorescent signal. This is because in practice the fluorescent dye of the antibodies in the solution is barely illuminated at all.

Another advantage is that there is room on the chip for several hundred spots, so that – providing there are suitable antibodies – up to 20 different mycotoxins can be measured at the same time in one process.

"We at Bayer CropScience and Bayer Technology Services are working in several teams: one group is concerned with the biochemical reaction and obtaining suitable antibodies, another optimizes the microliter-sized measuring chamber, whilst yet another is working on the precision of the spotter which applies the scavenger molecules," is how Burmeister describes the complex development process. "Others are concerned only with the read-out instrument, or the requisite production processes. One team measures the mycotoxin concentrations and compares them with the results obtained using the standard laboratory procedure, and last but not least, another group is preparing the market launch."

Once the system has been standardized, the user only has to carry out a simple cereal extraction and fill the measuring chamber. The read-out process then follows automatically. The product is expected to be available on the market within a few years. The project leader is convinced: "It is a genuine platform technology, the benefits of which are far from exhausted by food diagnostics."

www.zeptosens.com
Planar waveguide technology is explained on the website of this subsidiary of Bayer Technology Services.

Interview with:
Heiner Klasen,
Nörvenich (Rhineland)

Quality standards are constantly increasing

Heiner Klasen is the Cereals Department Manager at Buir-Bliesheimer Agrargenossenschaft eG in Nörvenich in the Rhineland and is an expert in cereal storage and logistics. *research* talked to him about the importance of mycotoxins.

Mr. Klasen, are mycotoxins an important topic in the agricultural industry?
The subject of mycotoxins dominates all conversations and negotiations before every new harvest. Cereals with too high a deoxynivalenol (DON) content have currently almost no chance of selling. With EU Regulation 856/2005 coming into force on July 1, 2006, we now have limits for the DON and zearalenone (ZEA) content in cereals for the first time. We have to assume that the mills and concentrated feed plants, when buying cereals, will try to go much lower than this. Already, cereals with lower levels are much in demand in certain segments such as muesli.

How do you ensure compliance with the limits at present?
Firstly, we take samples before the harvest which will be tested in order to obtain a reference point for the level of mold infestation. Further checks are then carried out when these results have been obtained, followed by more intensive laboratory tests at a later date. At present, we are unable to effectively prevent contaminated and uncontaminated cereals being mixed together when put into storage.

Would you be interested in a mycotoxin diagnostic system?
We are very interested in a new, safe and quick mycotoxin diagnostic technique, as our customers' quality requirements are constantly rising. From the perspective of product liability, we also urgently need better controls and improved safety.