ImmuStick – novel rapid test for identifying disease pathogens

In some situations such as in the food and pharmaceutical industries or hospitals, a test to identify disease pathogens can be vital. At present, such tests are relatively time-consuming and can take hours or even days to produce results, depending on the pathogen. Scientists from the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB in Stuttgart are currently developing a rapid test called ImmuStick which is as easy to use as a pregnancy test and delivers the result in just a few minutes.

The ImmuStick can be used to identify bacteria, fungi, viruses or their pyrogenic components. The stick could potentially be used for identifying bacterial contaminations in drinking water.

The human immune system usually ensures that disease-causing intruders such as bacteria, viruses or fungi are recognised and rendered harmless as quickly as possible. Small pathogen fragments, so-called pyrogens, are all that is needed to trigger an immunological reaction that causes fever and inflammation. The receptors of the innate immune system are responsible for inflammatory reactions as they are able to recognise certain structures, i.e. those typically seen in bacterial membranes. However, pyrogens sometimes manage to enter the bloodstream, for example in weakened intensive care patients, where they cannot be controlled adequately. Pyrogens in the blood often lead to sepsis or blood poisoning, which causes an estimated 18 million deaths every year.

It is therefore important to test medical products, pharmaceuticals, the surface of medical devices, drinking water and food, as these situations all require a complete absence of pyrogens. Currently available tests are relatively time-consuming and limited to certain pathogens. In addition, identifying pyrogens requires appropriate laboratory equipment and animal experiments. These are time-consuming procedures and it can take several hours or even days after the withdrawal of blood before results are available.

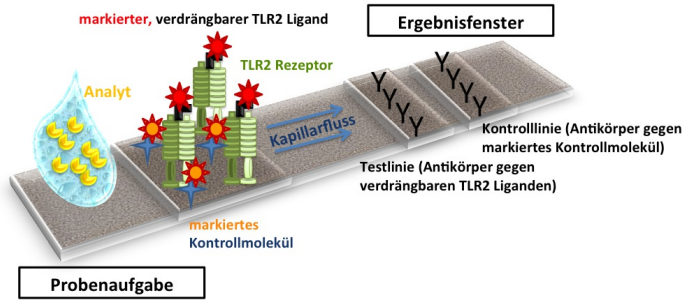
Immuno-based tests identify intruding microorganisms

For these reasons, the Molecular Cell Technologies group at the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB) in Stuttgart has been focusing in recent years on the development of alternative in vitro test systems based on innate immune system receptors. The researchers are particularly focused on so-called Toll-like receptors (TLRs), which detect foreign substances after they have entered the body and initiate the production of fever-triggering substances. The Fraunhofer IGB group of researchers already developed and patented a cell-based test called a PAMP assay based on these human immune receptors around ten years ago. PAMPs (pathogen-associated molecular patterns) are isolated highly conserved structures in many microorganisms and pyrogens (e.g. lipopolysacchrides in the cell walls of bacteria).

“The PAMP assay is a valuable and reliable tool that we have been using on a regular basis for screening drugs or detecting fever-causing substances. These investigations were mainly carried out on behalf of other companies,” said Dr. Anke Burger-Kentischer, a cell biologist who heads up the group of IGB researchers. “Unfortunately, the test is quite time-consuming and it takes around two days for results to be available. We therefore wanted to simplify the whole procedure and immobilise immune receptors on test strips.” Burger-Kentischer had had this idea in mind for quite some time, but did not have the time or money to take it any further. The ImmuStick project has since been supported with financing from the Fraunhofer Society’s Discover programme that funds projects aimed at demonstrating the feasibility of a particular technology.

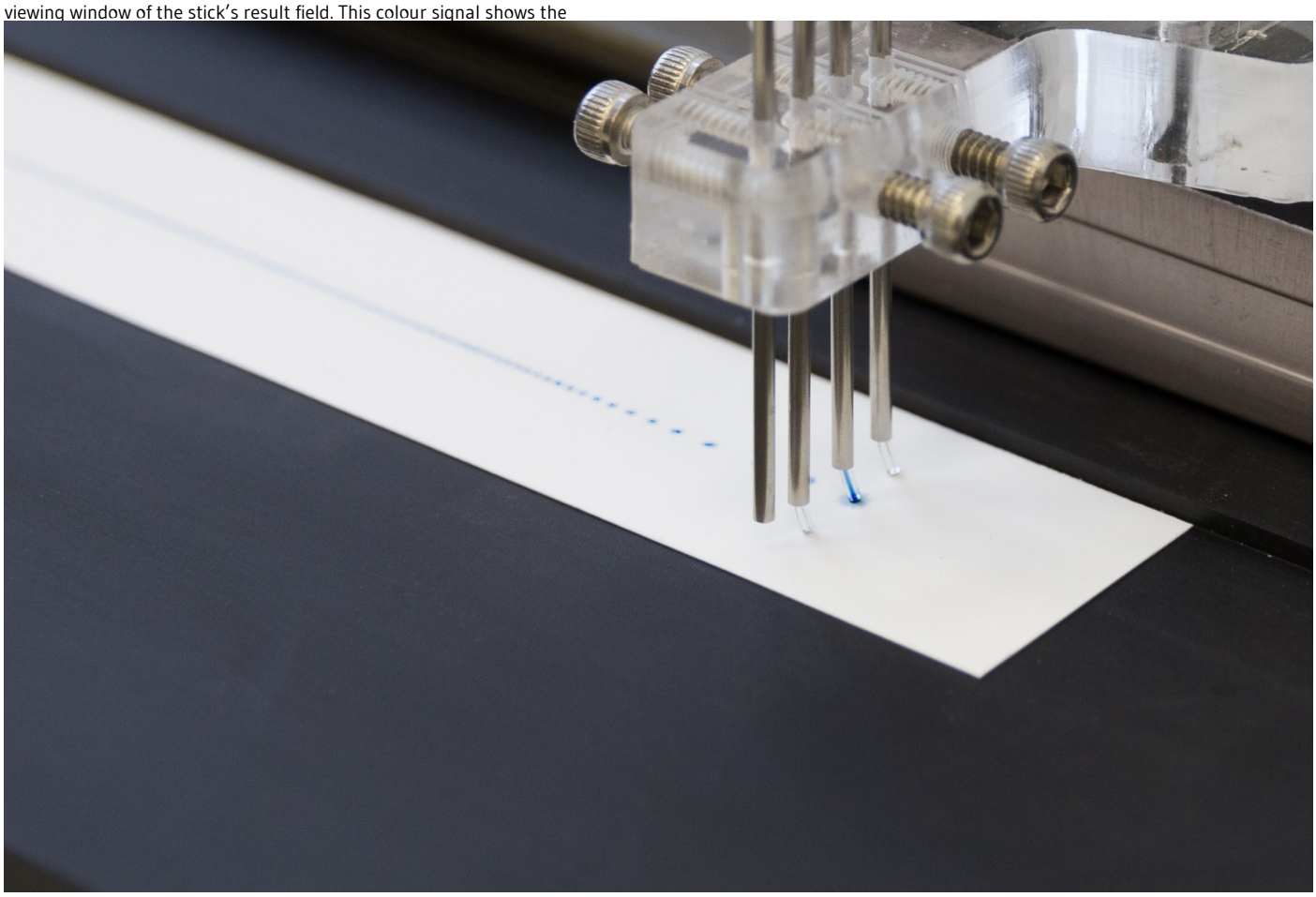
“Pregnancy test” for disease pathogens

This led to the idea of developing the ImmuStick, a rapid test for identifying pyrogens, which works just like a pregnancy test. Components of the innate immune system are immobilised on a paper-based test stick. A few drops of sample fluid are applied to a specific spot on the stick. If the sample contains pathogens or pathogen fragments, a coloured strip appears in the viewing window. “A biochemical reaction takes place between the sample and result fields. The biochemical reaction takes up to, but no more than, ten minutes, after which investigators are able to tell with the naked eye whether the sample contains something that can cause fever,” says Dr.-Ing. Christina Kohl who has been involved in the development of the 7-cm-long, and 0.5-cm-wide ImmuStick. “No special laboratory equipment or cooling are required for the test. However, the stick is sensitive to humidity,” says Christina Kohl.



Receptors of the innate human immune system that are sensitive to certain pyrogens are immobilised on the surface of the stick in order to generate the biochemical reaction. The receptors act as capture molecules for a pyrogen of interest, and are synthesised in the laboratory on the basis of the biological model. At the immune-receptor docking point to which the pyrogens normally bind, a type of placeholder marked with a dye is mounted. When drops of a fluid that contains pyrogens are applied to the stick, the pyrogens displace the

placeholders, which have a much lower receptor-binding capacity than the pyrogens. “The colour-labelled placeholders are released and migrate with the fluid through the test strip until they are visible as coloured stripes in the viewing window of the stick’s result field. This colour signal shows the



Production of the ImmuStick: application of immune receptors to the surface of the test strip.
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presence of pyrogens in the sample. “In the result window, a coloured control line also needs to appear. This shows that the test has worked, i.e. that a sufficient amount of fluid has been applied,” says Kohl. “This is particularly important when the test is negative.”

Identifying disease pathogens in all kinds of samples

The test works with all kinds of liquid samples. “The test works with drinking water, injection solutions, basically everything that can enter human beings,” said the scientist. “However, the test is also suitable for solid medical products that can be dissolved, or for surface samples that can be washed off. We have already examined a large number of samples with our cell-based PAMP assay – liquid ones, solid ones that can be dissolved and solid ones that cannot be dissolved. All of them worked well.”

The test would also be of interest for analysing human body fluids such as saliva, urine and blood. The young engineer adds: “These are complex fluids with numerous different components. So we need more time to develop test systems suitable for this kind of fluids. However, a few technical solutions are already on the market, and could be used for this purpose. So we are aiming at this type of analyses in the medium term.”

Flexible test strips recognise several pathogens simultaneously

However, the Fraunhofer researchers are still working on the development of the first, simpler version of the test strip. “We already have a prototype,” says the scientist. “Unfortunately, we do not yet have any cooperation partners to help us bring the test to market quickly. However, there is a lot of interest in the test system.”

Schematic drawing of the ImmuStick.
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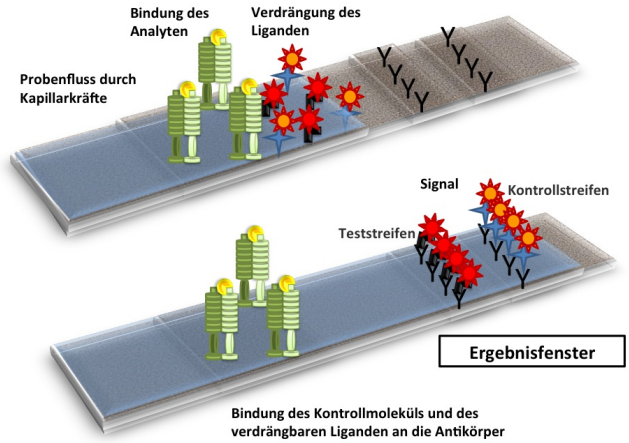

Functional principle of the ImmuStick after a sample has been applied (pyrogen is shown in yellow)

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The researchers have immobilised Toll-like receptors (TLR4) which recognise gram-negative bacteria on the test strip prototype. “The system is very flexible, and can be extended with other receptor subgroups,” says Kohl. “We started off with TLRs for historical reasons, but whole test strip sets or multiplex test strips that could be used to identify bacteria in general – both gram-negative and gram-positive ones – on the same stick work just as well.