

- 1 Schematic diagram of ImmuStick.
- 2 Fluorescence photometric evaluation of TLR4 bound to agarose beads with ligands (see Fig. 3).

IMMUSTICK – INNATE IMMUNE SYSTEM AS TEST STRIP

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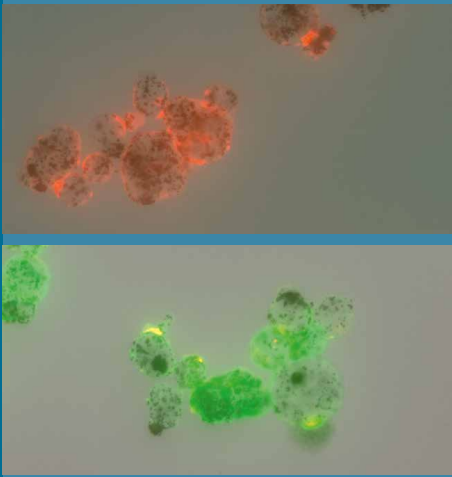
The Challenge of Sepsis

Around 18 million patients worldwide die from sepsis every year. This is caused by bacteria, viruses or fungi or their pyrogenic residues – that enter the patient's bloodstream. Pyrogens (Pathogen-Associated Molecular Patterns, PAMPs) are recognized by receptors of the innate immune system (Pattern Recognition Receptors, PRRs), which induce the production of fever-inducing messenger molecules.

Conventional sepsis diagnostic are time consuming and they are limited to live pathogens that can multiply. In order to prevent pyrogens from entering the bloodstream via medical devices and pharmaceuticals, they have to be tested for the absence of pyrogens. Four commercial detection systems are currently approved. These are either very expensive or limited to certain pyrogens.

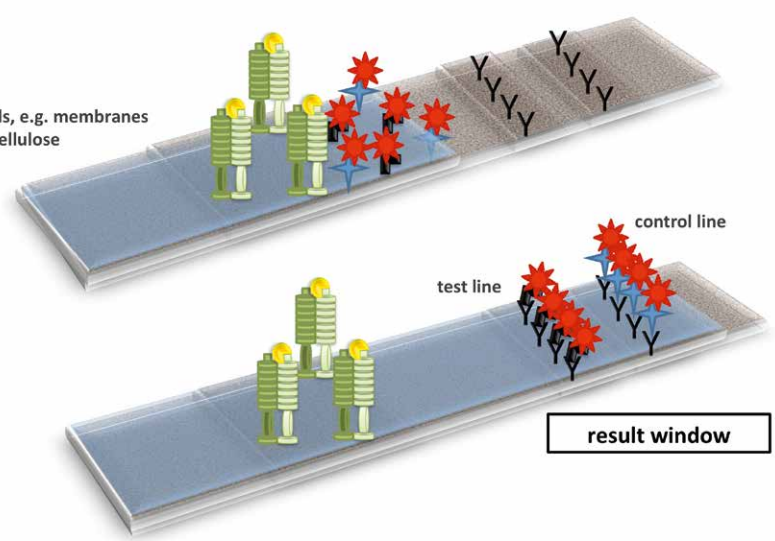
Pyrogen detection using immune receptors

For this reason, the Fraunhofer IGB has been working for several years on the development of alternative *in vitro* test systems based on the use of PRRs. Toll-like receptors (TLRs) represent the largest and best-known family of these PRRs. A sensitive and universally adaptable *in vitro* method is the PAMP assay (DE 10 2006 031 483; EP 2 041 172) patented by the Fraunhofer IGB. The test system is based on mammalian cell lines which stably express TLR and specifically indicate the presence or absence of pyrogens using a reporter gene. However, this method also requires a well-equipped laboratory and know-how in the handling of cell cultures.



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solid pads, e.g. membranes of nitrocellulose



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ImmuStick – the “pregnancy test” for pyrogens

We are developing an unconventional, novel detection system for pyrogens that uses PRRs as biosensors, without the use of animal experiments or time-consuming and expensive blood tests: the so called ImmuStick (DE 10 2014 223 430; EP 2 016 032 7580 patented by the Fraunhofer IGB). Based on the principle of immunochromatographic tests such as the pregnancy test, pyrogens are simply detected using a test strip. We use immobilized receptor domains of individual PRRs as binding molecules for the corresponding pyrogen. If an applied sample contains the corresponding pyrogen, a labeled ligand is released which indicates the presence of the pyrogen (Fig. 1).

Functionality of the ImmuStick

The analyte solution migrates from the sample application area via capillary flow into the biosensor area with immobilized TLR4 receptors to which labeled ligands are bound for the detection of pyrogens. Further there are labeled control molecules. In the area of the result window, antibodies are immobilized as capture molecules on a carrier matrix.

The detection of the pyrogen is based on a classical, competitive immunoassay. After wetting the test strip with the analyte solution, the pyrogen contained therein (TLR4 ligand LPS; yellow) displaces the labeled, weaker binding, labeled ligand (black-red).

Together with the control molecules (blue-red), these migrate to the result window in which specific antibodies against the labeled ligands are immobilised as capture molecules (Y). If the antibodies bind their antigen (the labeled ligand or control molecule), the application of a pyrogenic sample solution results in two coloured test lines in the result window. The control line verifies a functional sequence of the test (Fig. 2.).

Further application-specific development

In principle, we have demonstrated the feasibility of TLR4 for the detection of lipopolysaccharides (LPS). The test system can be extended modularly by receptors of the innate immune system (TLR, NOD-like receptors), C-type lectin receptors) in order to adapt the pyrogen spectrum specifically. Equipped with different PRRs, the ImmuStick can detect the entire range of PAMPs quickly, easily and directly on site.

Field of application

The ImmuStick, which is designed as simple point-of-care test, is suitable for the legally required testing for pyrogens in biological products and via sampling of medical devices. An application for the classification of sepsis pathogens directly on the patient is also conceivable. Since some PRRs also specifically detect allergens, the ImmuStick also offers potential for the determination of allergens.

- Pyrogen diagnostics for medical technology products (FDA guideline)
- Pyrogen diagnostics of pharmaceuticals, cosmetics and food
- REACH classification for chemicals
- Sepsis diagnostics
- Detection of allergens in medical technology, pharmaceutical industry and food technology

Advantages

The ImmuStick is the consequent future development of the patented cell-based *in vitro* immunoassay PAMP assay, where the immune receptor as biosensors are immobilized onto a test strip. The easy handling technique of the ImmuStick for the detection of bacteria, viruses or fungi or their pyrogenic residues can be easily applied by laypersons. The use of receptors of the human innate immune system allows the detection of all pyrogen classes.

- Very fast diagnostics (< 10 min)
- Simple, cost-effective and reproducible immediate test
- On-site process without equipment costs, designed as point-of-care test
- No specialist knowledge necessary

- 3 *Fluorescence microscopy of TLR4 bound to agarose beads: displacement of a ligands (red) weakly bound to TLR4 by LPS (green).*
- 4 *Functionality of the ImmuStick after sample application (pyrogens in yellow).*