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1 *MagnetReader with intuitive touch display developed at the PGI 8, Forschungszentrum Jülich*
2 *MagnetReader in use for the examination of virus infected plants*

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MAGN-I-TEKT
MAGNETIC IMMUNODETECTION FOR MOBILE AND RAPID IDENTIFICATION OF PLANT PATHOGENS

Plant pathogens such as viruses and fungi have a severe impact on crops worldwide and lead to considerable economic losses of up to 30%. The early identification and quantification of crop pathogens is therefore vitally important to prevent the spread of such diseases by initiating suitable actions like the use of plant protecting agents. Collecting and analyzing random plant samples routinely for the appearance of certain pathogens is an appropriate strategy. Continuous sampling on the field enables an early identification of pathogenic infestation and allows to efficiently control the amount of plant protecting agents.

Currently plant pathogens can be detected by several analytical methods. Besides microbiological methods e.g. for the detection of several fungal pathogens, serological and molecular methods are of mayor interest. Routinely the enzyme-linked immuno-sorbent assay (ELISA), established approximately 30 years ago, and polymerase chain reaction (PCR) based methods are employed because of their sensitivity. The disadvantage of all these methods is that the farmer needs to collect plant samples and subsequently send them to qualified analysis laboratories. This is labour intensive, time consuming and expensive. Usually farmers try to avoid this effort and the costs so that in many cases analysis laboratories are not entrusted until first symptoms become visible. Typically the appearance of phenotypic symptoms is characteristic for the final stadium of an infection. Thus after completing the lab based analysis, which usually takes several days or even weeks, the disease will have spread comprehensively.

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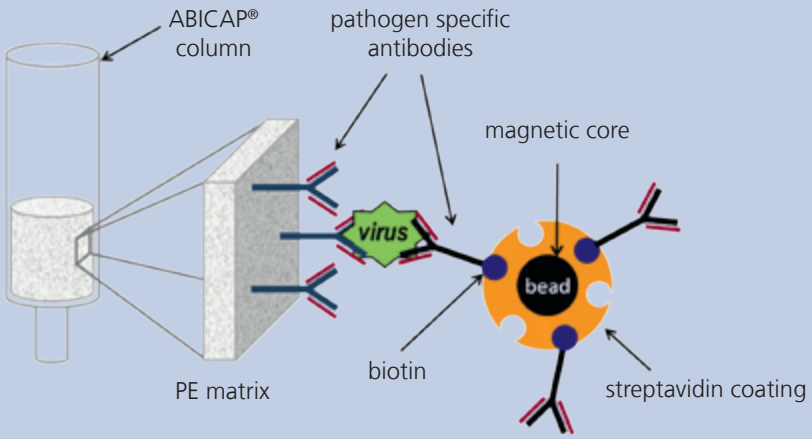

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Accordingly, there is great need in the development of a sensitive and inexpensive test that can be applied directly in the field and which identifies infected plants and quantifies the grade of infection within minutes. Furthermore the farmer needs to get precise advice from the measuring results regarding potentially required protective procedures.

Hence, the Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME) in cooperation with the Peter Grünberg Institute 8 at the Forschungszentrum Jülich has developed a new detection system allowing to analyze the grade of pathogenic infection directly in the field. Pathogenic infestation is detected indirectly by specifically modified magnetic nanoparticles and antibody-antigen interactions.

The detection unit developed at the PGI 8 – the MagnetReader – is based on the frequency mixing technology. High and low frequent magnetic fields are induced by different excitation coils. The sample carrying the magnic particles is placed within the resulting mixed field where it influences the frequency depending on the concentration of magnetic particles. The resulting frequency is detected by a pair of detection coils and the measuring signal is displayed.

In a first study at the Fraunhofer IME we successfully adapted the new detection principle for the detection of a plant virus. In grapevines infected with the *Grapevine fanleaf virus* (GFLV) high vintage losses of up to 80% are observed.

For the detection of this virus, GFLV specific antibodies were developed at the IME and have been produced by a hybridoma cell line. For the novel detection system these monoclonal antibodies were immobilized to a polyethylen matrix inside of an ABICAP® immunofiltration column. Such prepared these columns are employed to filter 0.5 mL of the plant extract that shall be analyzed. Thereby virus particles bind specifically to the antibodies inside of the column. Unbound plant material is removed by a washing step. Subsequently a biotin functionalized secondary antibody is added which also binds to the virus particles in the column. Finally streptavidin coated magnetic nanoparticles are rinsed through the column where they bind specifically and quantitatively to the biotin groups of the secondary antibody. Detection and quantification of the virus is achieved indirect via the magnetic particles deploying the detection coils of the MagnetReader. The measuring signal dependent on the infection grade is then displayed on an intuitive touch display.

Our ongoing research on the detection of GFLC revealed, that the assay is up to three times faster than a conventional lab-based ELISA. Furthermore we were able to increase the sensitivity by factor two. Consequently the assay allows the detection of just 0.5 ng virus per sample.
We plan to further optimize the assay by increasing detection speed and sensitivity. Additionally the assay will be adapted for the detection of a broad variety of different plant pathogens. The reader device will be modified in such a way that it can be used as handheld tool directly in the field. For this an easy and intuitive operation system is currently under development. Besides information about the kind of pathogen and the grade of infection the user shall also get information on the display regarding suitable counteractions.

1 *Immunofiltration column with bound magnetic particles are inserted into the Magnet-Reader for detection of pathogens*
2 *Schematic illustration of the detection principle inside of the immunofiltration column*