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Rapid DNA multi-analyte immunoassay on a magneto-resistance biosensor

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ABSTRACT

We present the rapid and sensitive detection of amplified DNA on a giant magneto-resistance sensor using superparamagnetic particles as a detection label. The one-step assay is performed on an integrated and miniaturized detection platform suitable for application into point-of-care devices. A double-tagged PCR amplification product of the *LamB* gene of the *Escherichia coli* bacterium was used to investigate binding kinetics of the assay. We applied magnetic actuation to concentrate the target–particle complexes at the sensor surface and to remove unbound particles from the sensor surface. We achieved biological dose-response curves detecting 4–250 pM amplicon concentrations in a one-step format in total assay times of less than 3 min. Using various tag-antibody combinations specific for one of the individual genes, multi-analyte detection is shown of several antibiotic resistance genes of the food pathogen *Salmonella*.

1. Introduction

Molecular diagnostic assays will increasingly be used in point-of-care settings in various areas such as genetic testing (Dobson et al., 2007) and for the detection of microbial pathogens and their antibiotic resistance (Robertson and Nicholson, 2005) in medical diagnostics, environmental control and food safety (Baeumner, 2003). The tests need to be cheap, rapid and simple to use, and in compliance with the required sensitivity and specificity. Preferably, the whole assay covers sample taking until the read-out of the results without any additional handling by the user. Therefore, miniaturization and integration of all assay steps into one disposable device is needed. The use of magnetic labels detected by a Giant Magneto Resistance (GMR) sensor is a powerful approach in this direction: actuation of magnetic particles speeds up the assay by reducing diffusion limitations, the absence of magnetic material in biological samples causes low background signals, and the stability of the detection labels and miniaturized biochip devices make the technology very promising for point-of-care settings. An overview of the current magneto-resistance sensor formats is given by Prins and Megens (2007). Several publications show the sensitive detection of magnetic particles, but publications describing real biochemical assays and target dose-response curves for nucleic acid testing are scarce. In particular, well-characterized biological limits of detection on magneto-resistance sensors have not yet been reported.

Detection of DNA hybridization with a GMR sensor was firstly described by Naval Research, using the Bead ARray Counter (BARC) for detection of biological warfare agents (Edelstein et al., 2000). Labeling of hybridized DNA targets with magnetic particles was done by settlement of beads on the microchip surface, after a more than 3-h hybridization step. Mulvaney et al. (2007) introduced force discrimination with a flowing wash fluid as a tool to improve assay specificity and sensitivity. In their paper, the dose-response curves were demonstrated on glass microscope slides rather than on magneto-resistance sensor chips. The Freitas group at INESC developed magneto-resistance based DNA chips using cystic fibrosis related DNA targets as a model system. Manipulation of magnetic labels by ac field focusing reduced the detection time to 15–30 min (Ferreira et al., 2005). The biological sensitivity of the system, i.e. the limit of detection of target DNA, was estimated by a theoretical calculation based on sensor response as a function of particle concentration, yielding a theoretical lower limit of 500 pM of target DNA (Ferreira et al., 2006). Schotter et al. (2004) determined the sensitivity of their magnetic biosensor by varying the probe concentration attached to the surface instead of the target analyte, using a 12-h hybridization step. Recently, they used non-specific magnetic particles as a mixing device in combination with a gradient magnetic field to reduce the hybridization time. However, fluorescent molecules instead of magnetic particles were used as a detection label (Heer et al., 2007).

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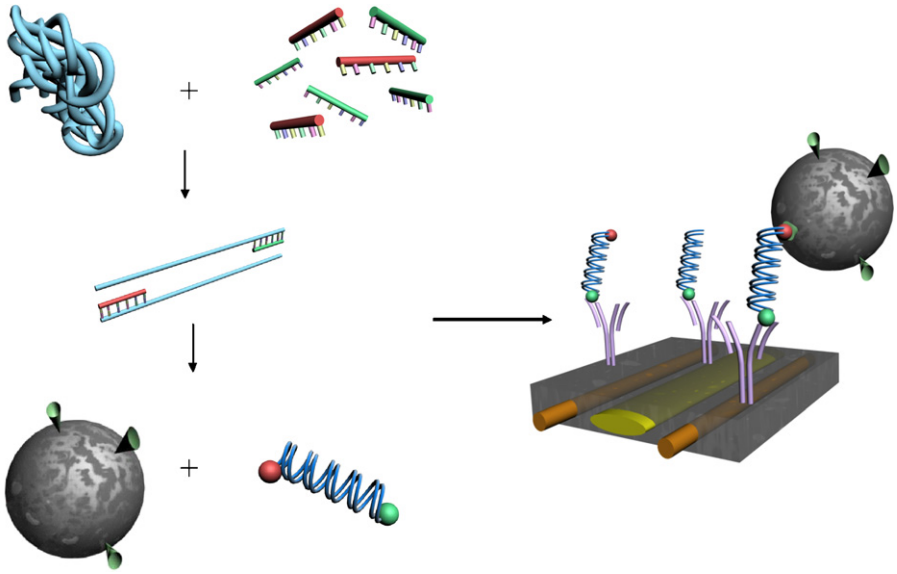

Fig. 1. Detection of double-tagged PCR product on a GMR sensor using magnetic particles in a one-step assay format. Amplification of an *E. coli* specific DNA product is performed by PCR on genomic DNA using a 5′-biotin forward primer and a 5′-fluorescein reverse primer. The double stranded-PCR product is mixed with streptavidin coated superparamagnetic particles and applied to the cartridge. Amplicon–particle complexes are captured by anti-fluorescein antibodies at the sensor surface and detected by the GMR sensor.

In Philips, a compact biosensor platform is being developed for the detection of biomolecules with superparamagnetic particles and magneto-resistance sensors having integrated field-generating wires (Prins and Megens, 2007). The silicon biochip is packaged in a disposable cartridge containing electrical connections for read-out electronics and a fluidic part to guide fluids over the chip (Nelissen et al., 2005). The excitation wires, generating the magnetic field to magnetize the surface-bound nanoparticles, have been integrated in the sensor chip. de Boer et al. (2007) developed a signal modulation scheme to achieve a high signal-to-noise ratio of the particles contributing to the assay signal compared to the noise of the detection system.

In this paper we describe the rapid and multi-analyte detection of amplified DNA by an integrated GMR biosensor. The nucleic acid detection is based on hapten labeling of amplicons and capturing by anti-hapten antibodies at the sensor surface. Such mixed immuno-DNA formats have been used in lateral flow and microfluidic detection assays (Kozwich et al., 2000; Corstjens et al., 2001; Baeumner et al., 2004; Koets and van Amerongen, 2005; Wang et al., 2006). In our assay, end-labeled primers are used to introduce tags during nucleic acid amplification; the specific amplicon is double-tagged with a biotin and a distinguishing tag such as fluorescein or digoxigenin. The resulting double-tagged amplicon is sandwiched between a corresponding antibody at the sensor surface and a streptavidin coated superparamagnetic particle (Fig. 1). We combine the advantages of magnetic labels, namely for particle movement by actuation and direct detection of the particle bound target, with the rapid kinetics of a tag-capture detection of nucleic acids. We use the pathogen *Escherichia coli* as a model to demonstrate biological dose-response curves in one-step and two-step assays. The two-step assay involves an incubation period with the DNA target and a fluidic wash of the sensor prior to the addition of magnetic particles. In the one-step format, the amplicons are mixed with the magnetic particles and immediately applied and detected by the sensor, resulting in a rapid detection method. The possibility to use the one-step format for multi-analyte detection is demonstrated by the multiplexed detection of four antibiotic resistance genes of the food pathogen *Salmonella* (van Hoek et al., 2005).

2. Materials and methods

2.1. GMR sensors and experimental setup

The silicon chip (1.4 mm × 1.4 mm, with a wetted surface of 0.7 mm × 0.7 mm) contains four single GMR sensors with integrated field-generating conductors. Each GMR wire (3 μm × 100 μm) is flanked by two excitation wires and these wires are integrated into the silicon of the biochip. Specifically bound superparamagnetic particles get magnetized when a current is applied through these wires. The in-plane component of the stray field detected by the GMR wire induces a change in resistance of the GMR, which corresponds to the amount of particles bound to the sensor. The biochip was assembled into a disposable cartridge and completed with electrical connections via a Molded Interconnection Device (MID) and a flex foil for connection to read-out electronics. Before attachment of the fluidic part to the MID, the sensor surface was functionalized with tag-specific antibodies. The composition and packaging of the complete GMR cartridge are described by Nelissen et al. (2005). The signal-to-noise ratio and stability of all four sensors on a chip were electrically determined. Cartridges with at least three high-performing GMR sensors were used in the biological experiments. The miniaturized reaction chamber above the sensor chip has a size of 1.2 mm × 1.2 mm × 0.4 mm, which corresponds to a volume of approximately 1 μl. In practice a somewhat higher fluid volume was needed (16 μl) due to the dead volumes in the tubing of the experimental setup.

GMR sensor signals were measured by a detection platform as described by de Boer et al. (2007). The detection platform generates an offset signal that is proportional to the sensitivity of the GMR sensor and is independent of the presence of magnetic particles. The offset signal results from the alignment of the current wires in relation to the GMR wire in the biochip, which is constant for each individual sensor. Therefore, the signal change due to particles is determined relative to the offset signal of the GMR prior to the introduction of magnetic particles. For actuation of the magnetic particles during the assay, the system is equipped with two electromagnets, one above and one beneath the sensor surface. Cur-

rents applied through these electromagnets evoke magnetic field gradients that were used to attract (bind) to or repel (wash) the particles from the sensor surface.

2.2. Functionalization of sensor surface

The sensor surfaces were coated with a gold/molybdenum layer. Prior to bio-functionalization of the sensor, the molybdenum layer was removed by etching with a solution containing H_3PO_4 (85%), CH_3COOH (100%), HNO_3 and H_2O (70:16:3:5). The biochips were thoroughly washed with water and isopropanol, followed by UV-ozone cleaning of the Au-layer for 10 min (UV-ozone photoreactor PR-100, UVP). Anti-fluorescein polyclonal antibodies (1 mg/ml; Zymed, San Francisco, USA) were adsorbed to the Au-layer on the sensor surface by incubating for a minimum of 2 h at room temperature. Excess antibodies were washed away with 0.1 M borate buffer (pH 8.8) after which the cartridges were closed by mounting the fluidic part to the MID with 180 μm PET double coated mounting tape (Nitto Denko, Japan). Subsequently the chamber was filled with 100 mM borate buffer (pH 8.8) containing 1% (w/v) BSA, incubated for 1 h at room temperature and stored at 4 °C until used.

For the multi-analyte detection assay, specific antibodies against the various tags were printed on the individual GMR sensors. Each cartridge contains four GMR sensors. Polyclonal antibodies against the specific tags texas red (TR, Molecular Probes, Paisly, UK), dinitrophenol (DNP, US Biological, Swampscott, USA), fluorescein (FITC, Zymed, San Francisco, USA) and digoxigenin (DIG, Roche, Basel, Switzerland) were diluted to 0.1 mg protein/ml in Phosphate Buffered Saline, followed by spotting on the Au-layer of the sensor surface with the MACH-2 printer developed by Philips Research and MiPlaza (Eindhoven, The Netherlands). This printer is equipped with an Autodrop AD-K-501 micropipette (Microdrop Technologies, Hamburg, Germany) with a 50 μm nozzle. Using the cross-shaped alignment markers on the sensor surface, the spots were printed onto the individual GMR sensors. Before printing, the droplet speed and the droplet flight path were measured. This information was used to correct for deviations and enhance the accuracy of the spot placement. Print settings used (voltage −62 V, pulse width 31 μs and printing frequency of 100 Hz) resulted in a droplet volume of 150 pl and a droplet speed of 1.5 m/s. The spot diameter was approximately 200 μm covering the whole sensor on each cartridge. Repeated printing of droplets resulted in a final spotted volume of approximately 1000 pl on each sensor. After application of the microfluidic unit to the MID, the cartridges were blocked in a 100 mM borate buffer (pH 8.8) containing 1% (w/v) BSA and stored at 4 °C until used.

2.3. Singleplex and multiplex amplification

As a model system for specific detection of amplicons by GMR, amplification of a coding region of the *LamB* gene of *E. coli* was chosen (Bej et al., 1990). The forward primer (FP) and reverse primer (RP) were end-tagged with fluorescein and biotin, respectively (5′-fluorescein-CTGATCGAATGGCTGCCAGGCTCC-3′ and 5′-biotin-CAACCAGACGATAGTTATCACGCA-3′) and purchased from Eurogentec (Seraing, Belgium). *E. coli* (O 157 type) was cultured in Luria Bertani medium overnight at 37 °C, followed by genomic DNA isolation using the Gen Elute Kit (Sigma, St. Louis, USA). The resulting DNA was used as a template for PCR. PCR was carried out using 30 ng template mixed with 25 μl RedTaq MasterMix (Sigma), 10 pmol RP and 10 pmol FP in a total volume of 50 μl. Amplification was done using a 9800 Fast Thermal Cycler (ABI, Foster City, USA) as follows: 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C. After 30 cycles, the mixture was kept at 72 °C for 7 min. The concentration of amplicons (345 bp) was determined using the 2100 Bioanalyzer in combination with DNA 1000 assay chips (Agilent, Amstelveen, The Netherlands).

Amplification of four different antibiotic resistance genes of *Salmonella* was used as a model system for multiplex detection. Primer development of these genes has been described by van Hoek et al. (2005). The primer pairs were composed of a forward primer with a specific tag and a reverse primer with a common biotin tag: tetracycline (5′-DNP-AAAGCCGGTTCGCATCAAAC-3′ and 5′-biotin-GGAAGATCGCATGTGTTGCC-3′), chloramphenicol (5′-FITC-CCTTCT-TCGTCTTCTTCTCG-3′ and 5′-biotin-GGTAGGATGAAGGTGAGGAA-3′), streptomycin (5′-TR-GCAGCGCAATGACATTCTTG-3′ and 5′-biotin-CATCCTTCGGCGCGATTTTG-3′) and ampicillin (5′-DIG-CGCTATCTGAAAATGAACCAG-3′ and 5′-biotin-TTTCGCTCTGCCATT-GAAGC-3′) (Eurogentec). The PCR reactions were carried out in a total volume of 50 μl containing 40 ng of *Salmonella* genomic DNA, 5 μl 10× AccuPrime PCR Buffer II, 10 pmol of forward and reverse primers, and 2.5 U of AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen, Breda, The Netherlands). Amplification was performed as follows: 30 s at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 55.8 °C and 1 min at 68 °C. After 30 cycles, the mixture was kept at 72 °C for 7 min. The PCR conditions for multiplex amplification were similar to the single reactions, except for the primer concentrations. Per reaction 20 pmol of forward primer mix, containing four tagged primers, and 20 pmol of reverse primer mix, consisting of the four reverse primers, was used. The resulting PCR products were analyzed with an Agilent 2100 Bioanalyzer using the DNA 1000 kit.

2.4. DNA immunoassay

Streptavidin coated magnetic particles (Ademtech, Pessac, France) were washed and diluted to an end-concentration of 0.1% (w/v) in 100 mM borate buffer (pH 8.8) containing 1% (w/v) BSA and 0.05% (v/v) Tween 20. The cartridge was washed with 100 mM borate buffer (pH 8.8) containing 0.05% (v/v) Tween 20 and connected to the detection platform. One-step and two-step assays were performed on the biochip. A series of amplicon dilutions (4–1000 pM) was tested in the *E. coli* dose-response curves. In *Salmonella* singleplex assays, an amplicon concentration of 500 pM was used. In the *Salmonella* multiplex assay, the sample contained 256 pM DNP, 434 pM DIG, 449 pM FITC and 126 pM TR amplicons, as determined by the Bioanalyzer. Note that the concentrations are unequal due to yield differences of the individual amplification reactions during multiplex PCR. In case of the one-step assay, magnetic particles were mixed with the PCR product for 1 min and thereafter applied to the cartridge for magnetic actuation and detection. In the two-step assay, antibody-coated sensors were incubated with DNA amplicons for 30 min at room temperature. Thereafter the sensors were washed with 100 mM borate buffer (pH 8.8) containing 0.05% Tween 20. Finally, the magnetic particles were applied with the same actuation and detection procedures as in the one-step assay.

3. Results

3.1. Actuation of magnetic particles

A tag-capture assay of the double-tagged *E. coli* PCR product was used to determine the biological performance of the GMR sensor. Each of the strands of the amplicon was 5′-end tagged witch fluorescein and biotin, respectively. The tag-captured amplicon detection is based on sandwiching the amplicon between the capture-antibody functionalized sensor surface and streptavidin coated magnetic particles. Superparamagnetic nanoparticles varying in diameter were tested for their suitability as a magnetic label.

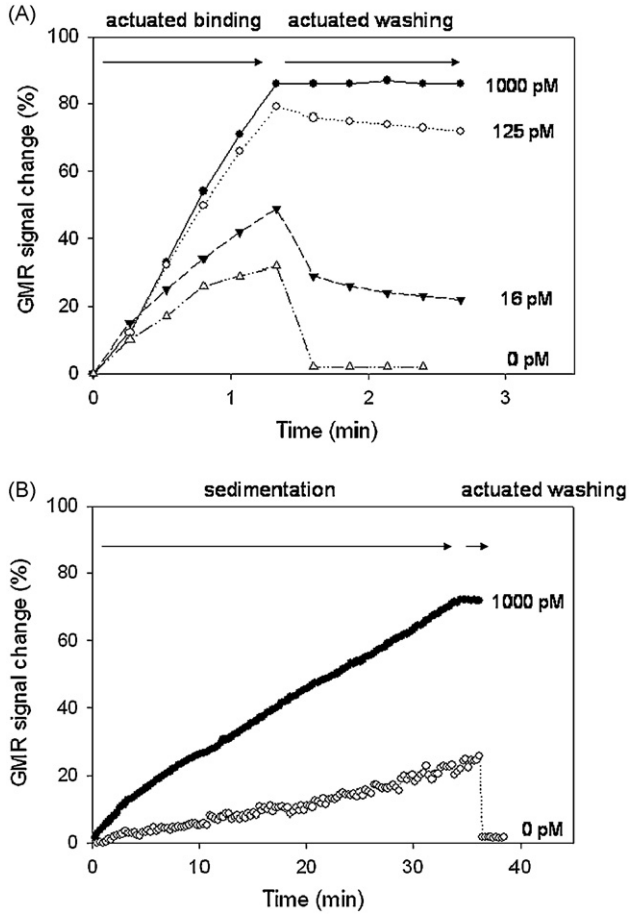

Fig. 2. Time trace of a one-step assay comparing (A) magnetic actuation with (B) sedimentation/diffusion of magnetic particles. At $t = 0$ the sample and magnetic particles were injected. For 1000 pM *E. coli* PCR product, an actuated assay takes 1.5 min. The same signal is reached in more than 30 min in a sedimentation/diffusion assay.

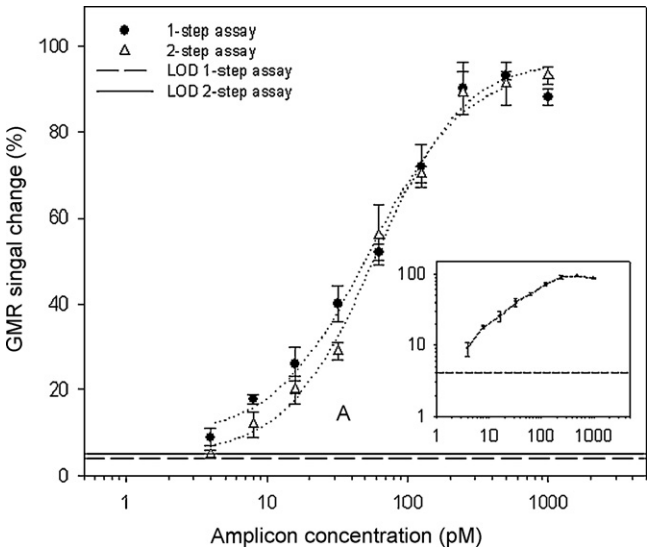

Fig. 3. Dose-response curves of a one-step and two-step assay. Different concentrations of FITC-biotin tagged *E. coli* PCR products were applied to the GMR cartridges. A negative PCR (i.e. no template) product was used as blank. The limit of detection (LOD) of the assays was the GMR signal change of the blank plus two times the standard deviation, resulting in a LOD of 5% for the two-step assay and 4% for the one-step assay. The error bars represent the standard deviations of signals measured on the different sensors of a single GMR cartridge. Dotted lines are guides to the eye. The insert shows the dose-response curve of the one-step assay on a log–log scale, in order to highlight the behaviour of the assay at low amplicon concentrations.

Smaller particles give lower steric hindrance and lower non-specific forces, while bigger particles have a higher magnetic moment and therefore are manipulated and detected more easily. Considering this balance, particles of 300 nm in diameter were found optimal, small enough for the biological assay and large enough for manipulation and detection.

Actuation of magnetic particles speeds up the assay kinetics by concentrating the particle–target complexes at the sensor surface, where binding to the capture antibodies can take place. Fig. 2 illustrates the influence of actuated concentration of superparamagnetic particles on the assay speed. The amplicon was incubated with the magnetic particles for 1 min, followed by injection into the microfluidic chamber of the biochip. A time-trace of GMR detection of four amplicon concentrations is plotted (Fig. 2A). The GMR signal change as a result of particles bound to the surface was measured every 13 s. After five steps of magnetic attraction, a magnetic wash was performed. In case of the blank (0 pM amplicon, i.e. PCR without template), the signal dropped to almost zero, indicating no binding of particles. For the 1 nM amplicon solution, specifically bound particles resulted in a GMR signal change of approximately 85%. When performing the same assay without actuation, i.e. using diffusion and sedimentation only, a comparable signal change is reached after more than 35 min (Fig. 2B). The signal during the magnetic washing process shows two phases: initially a rapid signal decrease and thereafter a stable signal with a very shallow slope. The rapid signal decrease corresponds to the removal of unbound and weakly bound particles. The stable signal corresponds to the more strongly bound particles. The slope of the stable signal is smallest for high amplicon concentrations, probably due to the strong attachment of the particles by multiple bindings per particle.

3.2. Biological dose-response curves

Biological sensitivity of the detection system was determined by applying serial dilutions of the *E. coli* PCR product in assay buffer containing 0.1% (w/v) end-concentration of streptavidin magnetic particles. Dilution series from 1 nM to 4 pM of amplicon concentration were tested in one-step and two-step assay formats. The results of at least three individual sensors per biochip were available for every data point; the means and standard deviations are depicted in the dose-response curves in Fig. 3. The background signal noise from the electronics and physical sensor was ±0.15% GMR signal change, as determined in experiments without magnetic particles, but otherwise with the same settings as in the assay. Down to 4 pM of amplicon could be detected with the two-step assay as well as with the one-step assay. Analysis of the biochips after the measurements by Scanning Electron Microscopy (SEM) confirmed the GMR signal responses. The complete chip surface was coated with antibody and, as a consequence, particles were able to specifically bind to the total surface area, as can be seen in Fig. 4. However, only particles binding at the actual sensing area ($1500\ \mu m^2$) contributed to the sensor signal change. For the higher analyte concentrations, sensors were fully covered with particles. Biochips of the blank samples showed very few particles, indicating that magnetic particles had bound in a biologically specific manner.

3.3. Multi-analyte detection

Four different PCR products corresponding to four antibiotic resistance genes of *Salmonella* were used for simultaneous detection by the GMR biochip. PCR amplification of genomic DNA with 5′-end tagged forward primers together with 5′-end biotin-

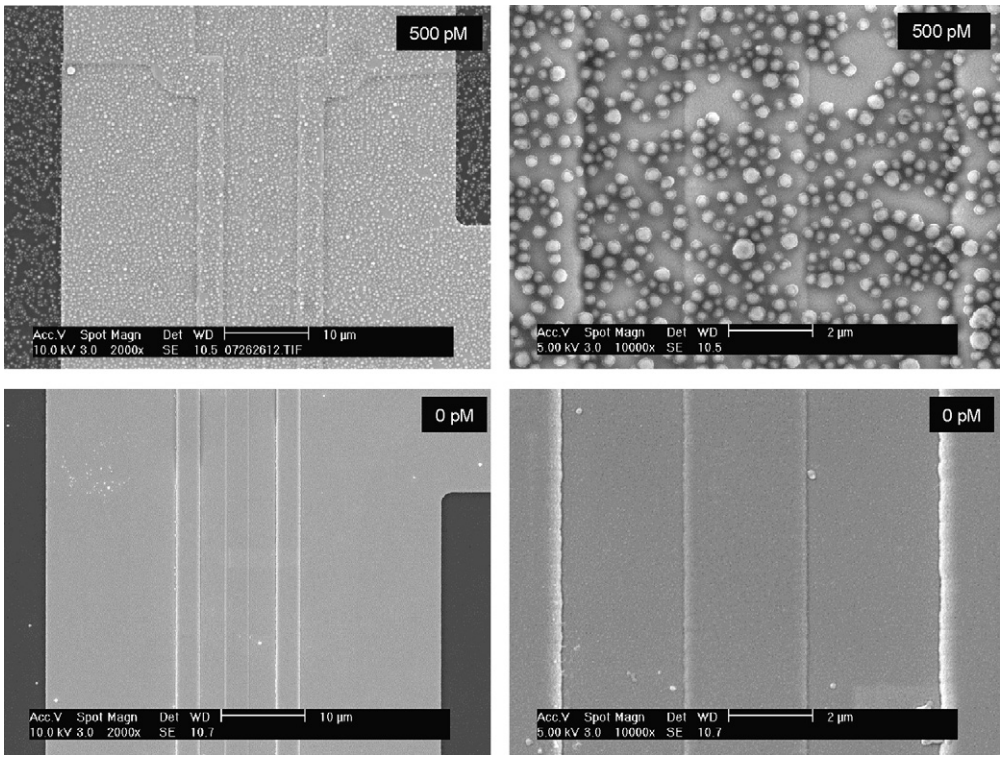

Fig. 4. SEM pictures of magnetic particles on the sensor after a one-step assay. In the upper images two different magnifications of one sensor are shown for a 500 pM PCR product. The GMR sensor is visible flanked by the current wires. The lower images were taken after a one-step assay with blank product (PCR without template).

tagged reverse primers resulted in double-tagged amplicons; one amplicon-specific tag for binding to the particular antibody printed at a GMR sensor and a common biotin tag to enable detection with streptavidin coated magnetic particles. The gene products were amplified in individual PCR reactions and in a multiplex PCR, i.e. the four primer sets were combined in one reaction. Specificity of the assay was tested by applying these individual and multiplex amplicons to GMR biochips, having each of the four sensors functionalized with one of the antibodies against a particular amplicon-specific tag. Assay conditions were the same as for the *E. coli* one-step assay, i.e., 1 min of target–particle binding, followed by injection to the cartridge and magnetic actuation and washing. GMR signals are presented in Fig. 5. Signals of the individual PCR products showed specific binding of the amplicons to their specific antibody-functionalized sensor and background signals were comparable with the blank of the *E. coli* assay. Only the FITC-tagged product showed a slightly higher background at the anti-DIG functionalized sensor. As expected, the multiplex PCR product gave a positive signal on all of the four sensors.

4. Discussion

The performance of this GMR platform for nucleic acid detection was evaluated using one-step and two-step tag-capture immunoassays. The sensitivity of the GMR system was determined by de Boer et al. (2007) in a two-step immunoassay. The number of particles on the sensor surface was determined by the GMR signal change as well as by optical imaging. It was shown that the GMR signal is linearly dependent on the number of particles on the sensor surface and it was derived that a density of three 300 nm particles on a sensor surface of 1500 μm^2 could be detected for a measurement time of 1 s. In this paper we investigate biological assays, with focus on the demonstration of dose-response curves and the limit of detection (LOD) in rapid assays. The biological LOD is stated to be the concentration at which the signal differs two standard deviations from the signal at zero concentration (the blank). For the two-step assay, the GMR signal change of the blank is 3% with a standard deviation of 1%. This results in a limit of detection at 5%

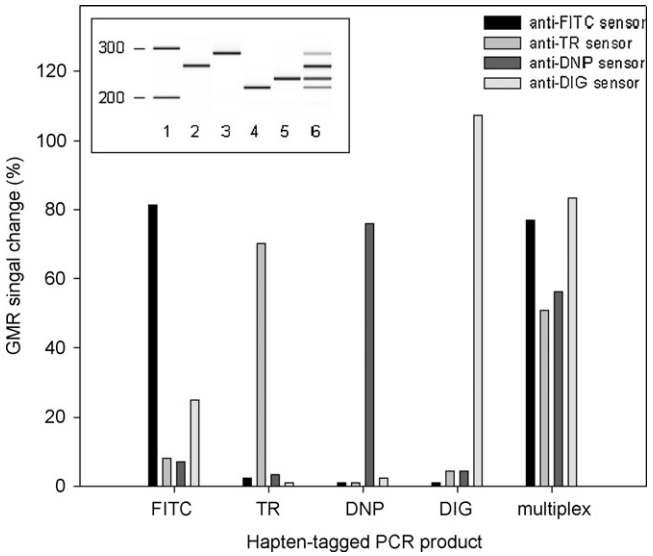

Fig. 5. Multi-analyte detection in a one-step assay format of *Salmonella* antibiotic resistance genes on antibody-printed GMR sensors. Differently tagged PCR products were applied to the cartridges, analyzing the specificity of each sensor for the corresponding amplicons. The insert shows the Bioanalyzer results of the single PCR reactions and the multiplex PCR, these fragments were used in the detection assay. Lane 1: base-pair ladder; lane 2: FITC-amplicon; lane 3: TR-amplicon; lane 4: DNP-amplicon; lane 5: DIG-amplicon and lane 6: multiplex PCR products. In the singleplex assays, the samples were diluted to an amplicon concentration of 500 pM. In the multiplex assay, the concentrations were: FITC 449 pM, TR 126 pM, DNP 256 pM and DIG 434 pM.

GMR signal change, which corresponds to a LOD of 4 pM of PCR product. The dose-response curves of the one-step and the two-step assay show that the LOD of both formats is comparable (Fig. 3). However, for the one-step assay the LOD corresponds to 4% GMR signal change, this is lower than the value of the lowest measurement point, which is 9% for 4 pM of amplicon concentration. So for the one-step assay the LOD is even lower than 4 pM of amplicon. Interestingly, in both assay formats the background signals (2% and 3%), the standard deviations, the LODs, and the concentrations of assay components are similar. However, an important difference between the one-step and the two-step assays is the total time required for the detection assay: 3 min for the one-step assay and 35 min for the two-step assay. To understand the difference in kinetics we need to consider the time-critical processes involved in signal generation, namely (i) the transport of target molecules from the solution to the sensor surface, and (ii) the subsequent binding of target molecules to the capture molecules on the surface. These processes are very different in the two assay formats. In the one-step format, amplicons are bound to the magnetic particles in solution and are then concentrated at the capture surface by magnetic actuation. In the two-step format, amplicons travel to the surface by diffusion only. Our results clearly show that the diffuse-and-bind process in the two-step assay is much slower than the catch-and-actuate-and-bind process in the one-step assay. Above 250 pM amplicon concentration, the assay signal saturates and in the one-step assay at analyte concentrations higher than 500 pM a decrease in signal was observed. This is typical for a solid-phase one-step sandwich immunoassay: at high analyte concentrations both capture and detection binding proteins become saturated with analyte, resulting in reduced bridge formation between these proteins by the analyte (the so-called high-dose-hook-effect). Experiments with multi-analyte detection of different gene products on one GMR biochip showed that similar signal changes and similar blank levels could be achieved as with the single analyte one-step assay (Fig. 5). The only exception is that the FTIC-tagged amplicons gave somewhat higher background signals, i.e. signals on antibodies other than anti-FITC. This points to a non-specific binding property of the FITC-tagged amplicon.

In our assay, the food pathogens *E. coli* and *Salmonella* were the starting material. Purified genomic DNA of these bacteria was used as a template for PCR and the resulting amplicons were applied to and detected by the GMR platform. Various groups have reported on the detection of specific DNA hybridization using magnetic particle labels (Ferreira et al., 2005; Mulvaney et al., 2007). However, in these studies synthetic oligonucleotides spiked into buffer solutions were used. None of these studies have been performed with natural biological samples in which target molecules need to be amplified to be detectable by the system and in which many non-complementary DNA strands are present that can interfere with the reactions. The observation of rapid detection of amplicons derived from natural biological samples makes the magnetic particle technology promising for application in a rapid point-of-care device. For example, on-chip PCR in combination with tag-capture detection of double-tagged PCR products has been described by Wang et al., 2006, in a microfluidic device with an integrated lateral flow strip. The benefit of amplicon detection by tag-based capture above hybridization of targets to a complementary probe on the surface, is at least two-fold: (i) antibody–antigen binding is faster than hybridization, and (ii) in hybridization, the complementary strand of the target strand competes with the capture probe for binding to the target strand, which reduces the binding efficiency.

5. Conclusions

In this paper we demonstrate the rapid and sensitive detection of tagged PCR amplicons on a giant magneto-resistance detection platform. Actuation of magnetic particles used as detection labels speeds up the total assay time resulting in a 3-min incubation and detection assay. The amplicons can be measured over two orders of magnitude, ranging from 4 to 250 pM of PCR product. Experiments have shown that the detection platform can be used for multi-analyte detection of four different amplicons with equal performance. We consider this rapid and sensitive detection system to be very promising for further integration into a point-of-care nucleic-acid analysis instrument, including miniaturized sample pretreatment and amplification.

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References

Baeumner, A.J., 2003. Anal. Bioanal. Chem. 377, 434–445.
Baeumner, A.J., Jones, C., Wong, C.Y., Price, A., 2004. Anal. Bioanal. Chem. 378, 1587–1593.
Bej, A.K., Steffan, R.J., DiCesare, J., Haff, L., Atlas, R.M., 1990. Appl. Environ. Microbiol. 56 (2), 307–314.
Corstjens, P., Zuiderwijk, M., Brink, A., Li, S., Feindt, H., Niedbala, R.S., Tanke, H., 2001. Clin. Chem. 47, 1885–1893.
de Boer, B.M., Kahlman, J.A.H.M., Jansen, T.P.G.H., Duric, H., Veen, J., 2007. Biosens. Bioelectron. 22, 2366–2370.
Dobson, M.G., Galvin, P., Barton, D.E., 2007. Expert Rev. Mol. Diagn. 7 (4), 359–370.
Edelstein, R.L., Tamanaha, C.R., Sheehan, P.E., Miller, M.M., Baselt, D.R., Whitman, L.J., Colton, R.J., 2000. Biosens. Bioelectron. 14, 805–813.
Ferreira, H.A., Graham, D.L., Feliciano, N., Clarke, L.A., Amaral, M.D., Freitas, P.P., 2005. IEEE Trans. Magnetics 41 (10), 4140–4142.
Ferreira, H.A., Cardoso, F.A., Ferreira, R., Cardoso, S., Freitas, P.P., 2006. J. Appl. Phys. 99, 08P105.
Heer, R., Eggeling, M., Schotter, J., Nöhammer, C., Pichler, R., Mansfeld, M., Brückl, H., 2007. J. Magn. Magn. Mater. 311, 244–248.
Koets, M., van Amerongen, A., 2005. In: van Amerongen, A., et al. (Eds.), Chapter in Rapid Methods for Biological and Chemical Contaminants in Food and Feed. Wageningen Academic Publishers, Wageningen, pp. 105–126.
Kozwich, D., Johansen, K.A., Landau, K., Roehl, C.A., Woronoff, S., Roehl, P.A., 2000. Appl. Environ. Microbiol. 66 (7), 2711–2720.
Mulvaney, S.P., Cole, C.L., Kniller, M.D., Malito, M., Tamanaha, C.R., Rife, J.C., Stanton, M.W., Whitman, L.J., 2007. Biosens. Bioelectron. 23, 191–200.
Nelissen, T., Weekamp, W., Van Delft, J., Ansems, W., Janssen, E., Prins, M., Megens, M., Wimberger-Friedl, R., Van Iersel, B., 2005. Proc. of the 15th European Microelectronics and Packaging Conference and Exhibition, Brugge, Belgium.
Prins, M.W.J., Megens, M., 2007. In: Buschow et al. (Eds.), Chapter in Encyclopedia of Materials: Science and Technology, Elsevier, pp. 1–6, doi:10.1016/B978-008043152-9/02146-1.
Robertson, B.H., Nicholson, J.K.A., 2005. Annu. Rev. Public Health 26, 281–302.
Schotter, J., Kamp, P.B., Becker, A., Pühler, A., Reiss, G., Brückl, H., 2004. Biosens. Bioelectron. 19, 1149–1156.
van Hoek, A.H.A.M., Scholtens, I.M.J., Cloeckaert, A., Aarts, H.J.M., 2005. J. Microbiol. Methods 62, 13–23.
Wang, J., Chen, Z., Corstjens, P.L.A.M., Mauk, M.G., Bau, H.H., 2006. Lab Chip 6, 46–53.