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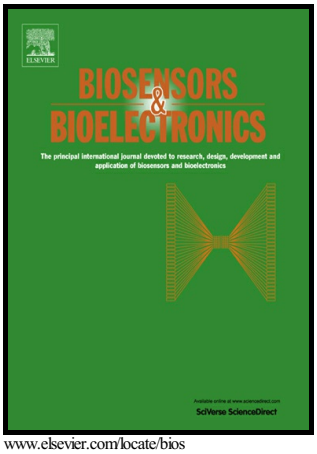

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Rapid dry-reagent immunomagnetic biosensing platform based on volumetric detection of nanoparticles on 3D structures

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Abstract

A dry-reagent immunomagnetic (DRIM) biosensing platform is developed for rapid high-precision quantitative analyses for *in vitro* diagnostics. The platform combines the advantages of immunochromatography with highly sensitive quantification of 200-nm magnetic nanoparticles (MP) from the entire volume of lateral flow membranes. The MP are registered by their non-linear magnetization at combinatorial frequencies by a portable reader that offers the detection limit of 60 zeptomoles or 0.4 ng of magnetic nanolabels and extremely wide linear dynamic range of 7 orders. The efficient combination of small MP with large reaction surface of the 3D membranes has permitted the detection in human serum of as low as 25 pg/ml total prostate specific antigen (PSA) during 30-min assay. The featured 3-fold signal increase per every order of concentration within 3.5 orders of magnitude allows precise analysis of antigen concentrations in a wide range. The system also provides the first tool for quantitative MP mapping along the lateral flow strip for easy development and optimization of assays. The DRIM detection can be used for simple, rapid and sensitive quantification of protein biomarkers for *in vitro* diagnostics both in laboratory and near-patient conditions, for food analysis, environmental monitoring, security, and safety applications.

Keywords

Magnetic particle quantification, biosensor, lateral flow, in-vitro diagnostics, prostate-specific antigen, non-linear magnetization

1. Introduction

Functionalized nanoparticles are of considerable interest for various biosensing and medical applications from the magnetic cell separation originated about four decades ago (Kronick et al., 1978) and using magnetic beads as solid phase in advanced bioassays (Haukanes and Kvam, 1993) to recent developments of the nanoparticles for drug delivery (Couvreur, 2013) and biocomputing nanostructures assembled as autonomous theranostic nanorobots (Nikitin et al., 2014). The biolabelled nanoparticles are promising for medical diagnostics *in vitro* and for control of therapeutic treatment by quantification of the disease protein markers in various biological samples such as blood, saliva, biopsy tissues, etc. (Doane and Burda, 2012). Among the most common approaches used for these purposes, immunoassays hold a special place (Chan, 2012). Continuous enhancement of their sensitivity and precision along with reduction of the analysis time (Shen et al., 2014) are highly demanded by healthcare. Magnetic nanoparticles (MP) employed as detectible labels offers substantial advantages over the optical markers commonly used in the immunoassays. The MP can be applied in non-transparent or strongly scattering media, e.g., in combination with 3D solid phases (Nikitin et al., 2007a, 2007b); exhibit high "signal-to-noise" ratio due to the absence of magnetic background in biological samples (Osterfeld et al., 2008); provide a stable signal not affected by reagent chemistry or photo-bleaching (Rife et al., 2003); can be manipulated with an external magnetic field (Miltenyi et al., 1990; Rida and Gijs, 2004; Aytur et al., 2006); can

enhance the efficiency and diminish the assay time due to "magnetic mixing", magnetic washing and separation.
The relationship between signal and quantity of magnetic labels deposited on the sensor surface is widely reported for various biosensors. However, very few of these reports present the dose-response curves over a wide concentration range of protein markers for *in vitro* diagnostics. The analysis of these studies shows that the magnetic label size influences the major assay parameters such as limit of detection (LOD) and dose-response sensitivity (slope of calibration curve). These parameters, in turn, strongly depend on immunoactive surface area of the used solid phase.
Relatively big submicron and micron (0.5 - 2.8 μm) magnetic labels are most popular. They can be easily detected on a flat 2D biosensor surface by giant magneto-resistance (GMR) sensors (Baselt et al., 1998), optical microscopy (Mulvaney et al., 2007, 2009), surface plasmon resonance (Krishnan et al., 2011), etc. These techniques demonstrate very attractive LOD up to the record attomolar values (Mulvaney et al., 2007, 2009; Krishnan et al., 2011). However, their dose-response sensitivity is still to be addressed as typically the signal rises by a small value of k_{log} = 1.15-1.5 times upon 10-fold increase of the antigen concentration. Many authors consider the assays that feature the low slope of calibration curve as suitable only for qualitative analyses of concentration by an order of magnitude (Mulvaney et al., 2009) or to obtain "yes/no" threshold readings because the error in signal is translated to the error in the determined concentration value. A few methods were tested for enhancement of the GMR signals by moving MP toward the optimal sensing area (De Palma et al., 2007) or by optical readout of magnetically actuated particles (Dittmer et al., 2010), which yielded the increased dose-response sensitivity. In those cases, non-trivial standardization of the dynamic parameters of the force applied to MP in different matrices may be among the factors to be dealt with for getting highly reproducible assays.
The nanoscale MP are considered promising for quantitative assays for *in vitro* diagnostics (Perrin et al., 1999; Gaster et al., 2009; Orlov et al., 2014, 2015) even on the limited surface of flat 2D biosensors because they substantially improve the dose-response sensitivity. For example, 50-nm MP provide doubling or tripling the signal upon increasing of antigen concentration by an order if recorded by the advanced GMR structures protected by an ultrathin 50-nm passivation layer (k_{log} = 2) (Gaster et al., 2009) or by the spectral-correlation interferometry (k_{log} = 3) (Orlov et al., 2014, 2015), respectively.
Much higher dose-response sensitivity k_{log} ~ 10 within the linear range of more than 3 orders is achieved with the same 50-nm MP detected by non-linear magnetization on a 3D solid phase (Orlov et al., 2013). This highly accurate quantitative immunoassay provides large reaction surface, quick reagent mixing, as well as antigen immunofiltration directly while the assay (Nikitin et al., 2007a; Orlov et al., 2013). The achieved LOD allows this method to replace the radioisotope-based assays for detection of protein markers of lung and bladder cancers, as well as for diagnostics of the fetal Down syndrome in clinical settings (Aldaz-Carroll et al., 2015). All the mentioned assays require liquid handling systems or development of reagent cartridges so their application beyond laboratory conditions is questionable.
The lateral flow immunochromatographic (IC) test is a much simpler dry-reagent format widely used with traditional color or fluorescent labels. For years, it was mainly utilized for threshold or semi-quantitative analyses that exhibited moderate LODs (O'Farrell, 2009; Posthuma-Trumpie et al., 2009). Recently, this format showed attractive parameters with MP used as color labels (Liu et al., 2011), as well as with the MP detection by planar coils of the MICT® reader (LaBorde and O'Farrell, 2002), non-linear magnetization (Nikitin et al., 2007a), resonant coil magnetometer (Barnett et al., 2014), by the GMR sensors (Taton et al., 2009; Marquina et al., 2012), etc. However, precise quantitative lateral flow techniques having analytical characteristics not worse than those of laboratory methods are still to be developed.

In this work, we have found an advantageous balance between the mentioned size-dependent peculiarities of MP and large reaction surface of the 3D immunochromatographic membranes (having the largest 260 μm thickness among the commercially available IC nitrocellulose) to develop a platform for rapid quantitative *in vitro* diagnostics, which offers the benefits of dry-reagent assays, provides attractive LOD in wide dynamic range using the MP labels readout from the entire volume of the membranes by the optimized non-linear magnetization technique (Nikitin et al., 2000a, 2000b, 2007a, 2007b). The developed Dry-Reagent ImmunoMagnetic (DRIM) platform has demonstrated the LOD in blood serum for a protein marker of prostate specific antigen (PSA) of 25 pg/ml, the dynamic range exceeding 3.5 orders and high dose-response sensitivity of k_{log} = 3. Along with easy storage of the dry-reagent kits, these characteristics of the platform permit robust quantitative near-patient measurements of both low and high analyte concentrations.

2. Materials and methods

2.1. Reagents

The following reagents were used in the experiments: PSA and mouse monoclonal antibody against PSA, clones P1 and P43 (Reagentika, Russia); rabbit anti-mouse IgG antibody (IMTEK, Russia); bovine serum albumin (BSA), Tween 20, phosphate buffered saline (PBS), carbonate-bicarbonate buffer (Sigma-Aldrich, Germany); N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, EDC (Fluka, Switzerland); N-hydroxysulfosuccinimide sodium salt, sulfo-NHS (Aldrich, USA); carboxyl-modified (COOH-) superparamagnetic particles (196 nm) (Merck Chimie SAS, France); female serum negative to PSA and ELISA kit (Reagentika, Russia). All the other auxiliary reagents were of "analytical grade" quality and were used without further purification. Deionized distilled water was used.

2.2. Preparation of conjugates of magnetic nanoparticles with antibodies

EDC (8 mg) and sulfo-NHS (4 mg) in phosphate buffer (10 mM, pH 5.0) were added to MP (0.3 mg) and incubated for 20 min under sonication. Three conjugates with different amount of cross-linkers added per 1 mg of MP were used: conjugate #1 – 2 mg EDC and 1 mg sulfo-NHS, conjugate #2 – 20 mg EDC and 10 mg sulfo-NHS, conjugate #3 – 50 mg EDC without sulfo-NHS. Then monoclonal antibody to PSA (35 μg) clone P1 in borate buffer (0.1 M, pH 8.6) was added to MP and incubated for 2 h in a horizontal shaker. After that, BSA (3.5 μl, 10%) was added and incubated for 30 min at permanent stirring. MP were twice magnetically washed out with PBS buffer (pH 7.4). The resulting particle concentration was adjusted to 10 mg/ml.

2.3. Fabrication of test strips

The assay was designed based on the IC lateral flow principle (O'Farrell, 2009; Posthuma-Trumpie et al., 2009) modified and optimized for using with 200-nm magnetic beads to be quantified by non-linear magnetization (Nikitin et al., 2000a, 2000b). The reagents were deposited onto membranes (UniSart CN140, thickness: 260 μm and 100 μm backing, kindly donated by Sartorius AG, Germany) with an automated dispenser "XYZ Platform 3060" (Biodot Inc., USA). MP-Ab conjugate was deposited onto the conjugation pad in concentrations 2.5, 5 and 10 μg/5 μl (5 μl onto membrane of 3.5 mm wide) and dried for 2 h at 37º C.

Monoclonal antibody to PSA (clone P43, concentrations of 0.25, 0.5, 1 and 2 mg/ml in borate buffer, pH 8.6) and rabbit antibody against mouse IgG (1 mg/ml in borate buffer, pH 8.6) were used to form on the nitrocellulose membrane the test and control lines, respectively. The density of antibody deposition was 2 μl per 1 cm of membrane width, the distance between the test and control lines - 12 mm. The resulting membrane was air dried at room temperature for 24 h. Then all the components of the IC test were attached onto a substrate and cut into 3.5 mm wide strips with an automated cutter "CM4000 Guillotine Cutter" (Biodot Inc., USA).

2.4. Statistical analysis

All the experiments were implemented at least thrice. In graphs, each value represents an average, and error bars show standard deviations. The limit of detection was defined using 2σ criterion as the minimal antigen concentration, at which the detectible signal exceeds the signal of negative control by at least two standard deviations of the negative control signal (Chaloner-Larsson et al., 1999). Correlation was analyzed by linear regression analysis and R2 was calculated (Motulsky and Christopoulos, 2004).

3. Results

3.1. Detection of magnetic nanolabels

The DRIM platform for *in vitro* diagnostics is based on using the magnetic nanolabels in the sandwich immunochromatography (Posthuma-Trumpie et al., 2009; O'Farrell, 2009), which are readout by the magnetic particle quantification (MPQ) method described in details earlier (Nikitin et al., 2000a, 2000b, 2007b). Briefly, the MPQ method employs a non-linear magnetization of particles subjected to a magnetic field at ac frequencies f_1 and f_2 with recording the particle response at a combinatorial frequency $f = n \bullet f_1 + m \bullet f_2$, where n and m are integers (one of them can be zero). The method is insensitive to linear dia- and paramagnetic materials. At the same time, its sensitivity to magneto-radioactive ^{59}Fe-based MP is on the level of the γ-radioactive technique (Nikitin et al., 2008). These features were successfully demonstrated for non-invasive *in vivo* MP mapping in organs of small animals, as well as for long-term study of MP evolution, clearance and redistribution in the animal's body (Nikitin et al., 2008, 2009).
In the present study for *in vitro* diagnostics, the test strips are passed through the measuring coil of the portable MPQ reader to quantify the number of magnetic nanolabels bound at the test line (TL) and control line (CL) (Fig. 1).

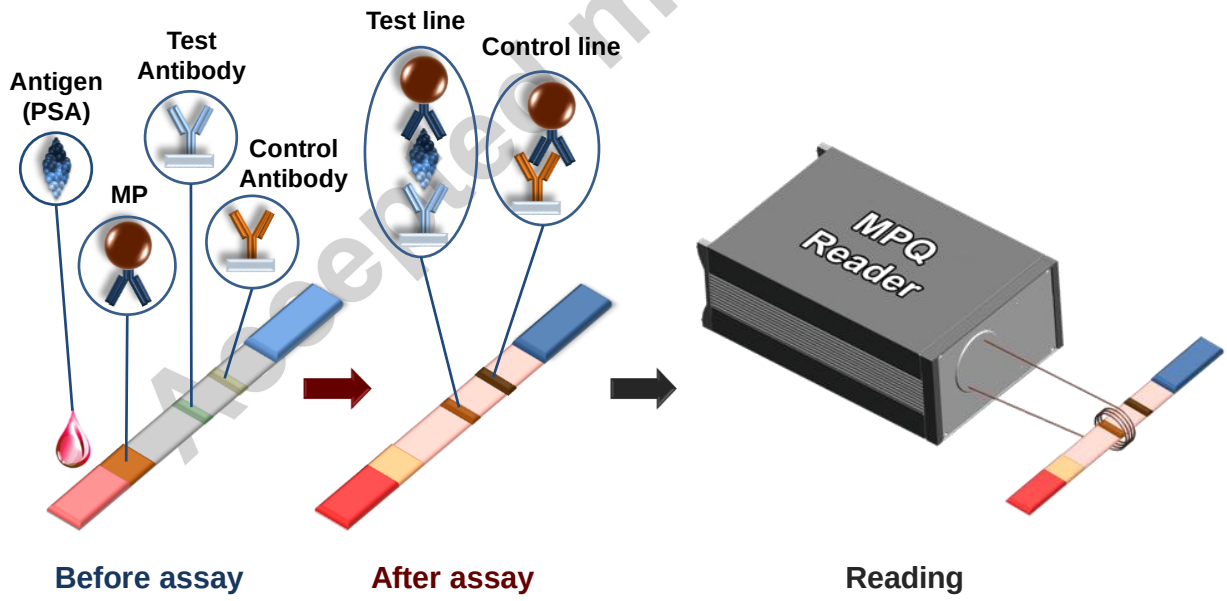

Fig. 1. Quantification of magnetic nanolabels in test strips: left - strip before assay; middle - strip after assay; right - quantification of nanolabels by an MPQ reader

In this research, we used the updated MPQ readers with zero drift of baseline and an order better limit of nanoparticle detection and 100-fold wider dynamic range as compared with the previous-generation readers (Nikitin et al., 2007b; Cherkasov et al., 2006) due to the increased

amplitudes of the low- and high-frequency components of the excitation magnetic field up to 144 Oe and 56 Oe, respectively. The limit of detection determined using the calibration curve shown in Fig. 2 is 0.4 ng of Fe_3O_4 nanocrystals in 0.2 ml volume with the dynamic range of more than 7 orders of magnitude. These parameters, to the best of our knowledge, currently have no analogs.

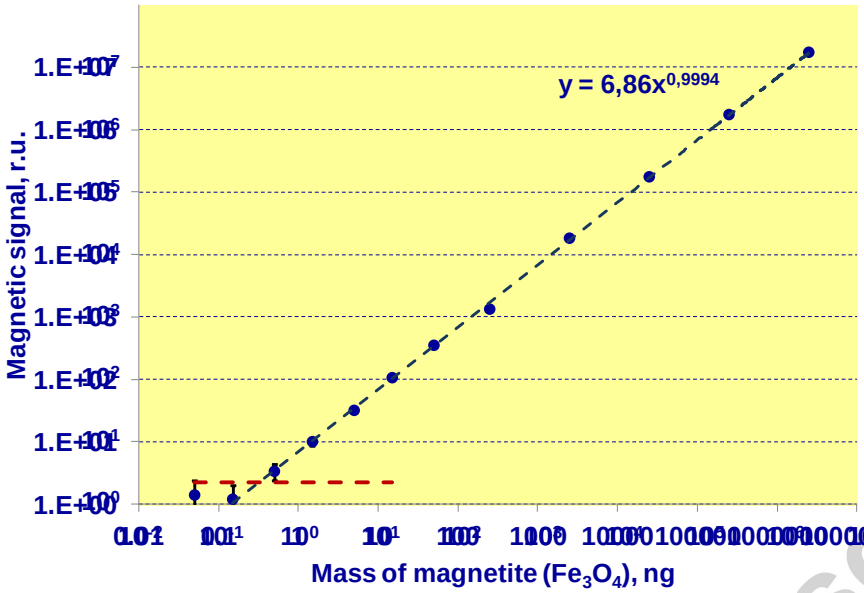

Fig. 2. Calibration curve of the MPQ reader signal vs. mass of magnetite

3.2. Characterization of components and optimization of parameters of the assay

The immunochromatographic membranes were characterized before and after the DRIM assay (Fig. 3). The membrane has enhanced surface with the pores of 5-30 μm (Fig. 3A). In the absence of antigen, the non-specific binding of MP with the membrane surface near the test line is negligible (Fig. 3B). The SEM image of the test line fragment taken after analysis of a sample containing 50 ng/ml PSA exhibits plenty of 200-nm MP specifically bound to the membrane (Fig. 3C).

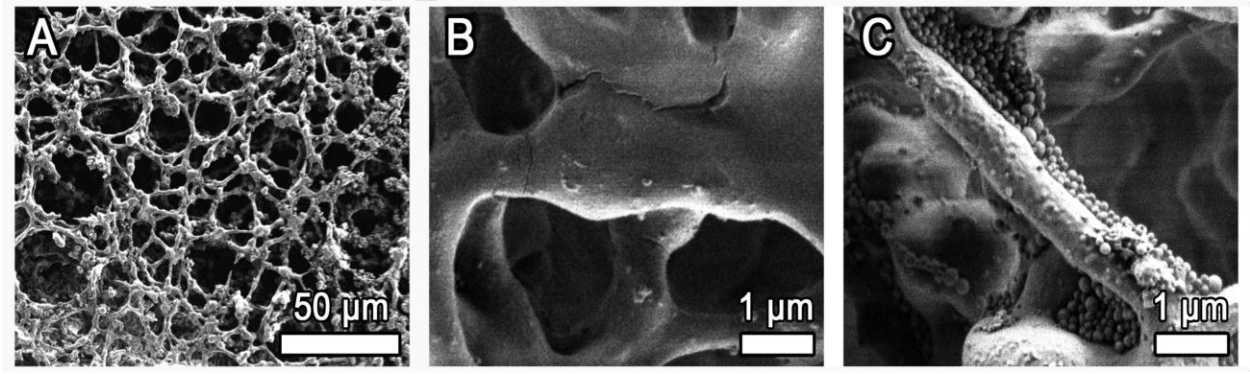

Fig. 3. SEM images of membrane at the test line: before assay (A); after analysis of a sample without PSA (B) and containing 50 ng/ml PSA (C)

To optimize the assay, we recorded the MP distribution along test strip under different conditions by continuous monitoring the magnetic signals while passing the strip through the MPQ measuring coil. Fig. 4 shows the results of such scanning for the samples containing 3 ng/ml PSA for the strips prepared with conjugates #1, #2 and #3, which differed in the amount of cross-linkers added during conjugation as described above. One can find 4 local

maximums in the MP distribution along any test strip at the: i) conjugation pad, ii) test line, iii) control line, and iv) absorption pad. The second maximum located at the test line is the most informative for determining the antigen concentration due to formation of specific "MP-Ab conjugate – PSA – detecting antibody" complexes. In Fig. 4, for example, the strips with conjugates #2 and #3 exhibit higher signal on the test line as compared to conjugate #1 so they are preferable for enhancement of the assay sensitivity.

We considered the portion of MP stuck on the conjugation pad area as a negative characteristic that pointed at low stability and reduced migration ability of the nanoparticles because they were not in fact involved in the assay. Indeed, it can be seen from Fig. 4 that the MP fraction on the conjugation pad was substantially lower when using conjugate #2 as compared with conjugate #3. Accordingly, conjugate #2 was chosen as the most favorable for the following experiments. Importantly, the integral of distribution dependence (area under the curve) is proportional to the initial amount of conjugate and is almost equal for all the three curves in the figure (see also Fig. S3). It should be noted that non-specific signals on the test line while testing the samples without PSA were virtually the same for the used three conjugates. So the results obtained with different conjugates were compared for the positive PSA samples. Other assay parameters such as the amounts of antibody and MP to be deposited onto the TL and conjugation pad, respectively, were optimized similarly (see Supplementary material). The optimized assay parameters are listed in the Experimental Section.

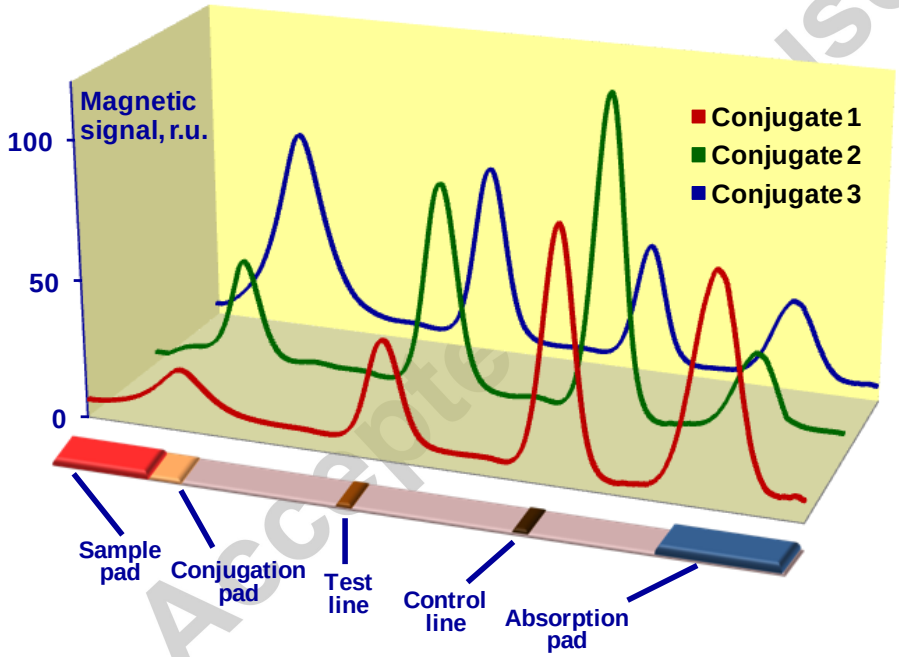

Fig. 4. MP distribution along test strip for the same 3 ng/ml concentration of PSA but with different MP-Ab conjugates prepared with various amount of cross-linkers.

3.3. Analytical characteristics of the DRIM platform

Under the optimized conditions, PSA was measured in undiluted blood serum using the DRIM platform. The studied 120-µl samples contained different PSA concentrations (0, 0.05, 0.1, 0.3, 1, 3, 10 and 50 ng/ml) spiked into female pooled serum negative for PSA. The magnetic signal distributions along the strips were recorded during 5 min (Fig. 5A). The signal from the test line was monitored for 30 sec with plotting the calibration dependence of signal against PSA concentration (Fig. 5B). The LOD determined by 2σ criterion (Chaloner-Larsson et al., 1999) for PSA was 25 pg/ml (or, by 3σ criterion, – 37 pg/ml) and the dynamic

range exceeded 3.5 orders of concentration. The signal at the control line is reliably registered (Fig. 5A) over the whole range of the used antigen concentrations. If necessary, the CL signal can be increased by applying larger amounts of the MP conjugate or other conjugate specific to antibody at the CL. The achieved wide dynamic range of 3-fold signal growth at every 10-fold concentration increase appears to be due to the optimized quantity of conjugates of small magnetic nanolabels registered from the entire volume of the membrane having enhanced immunoactive area.

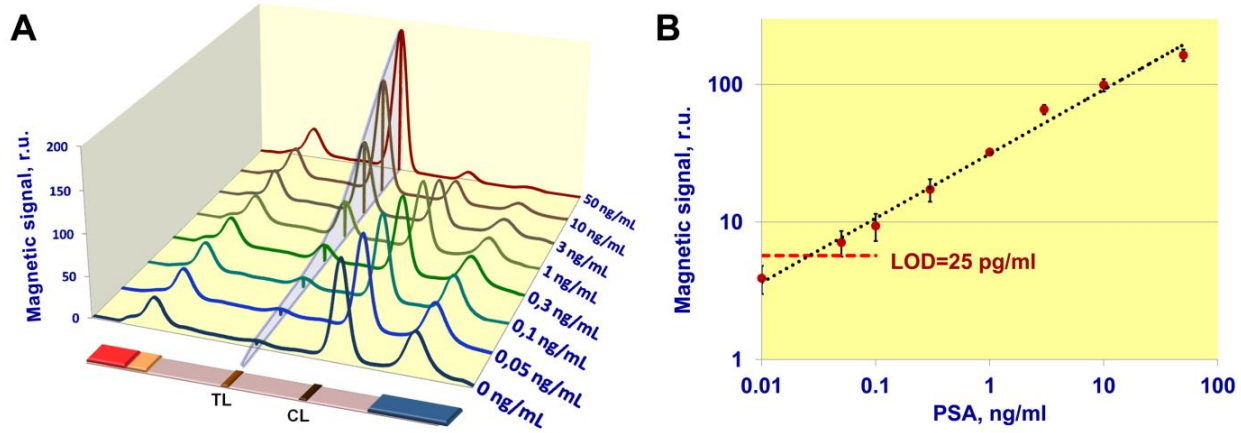

Fig. 5. Magnetic signals proportional to MP quantities: signal distribution along the test strips (A) and dependence of magnetic signal at the test line versus PSA concentration (B)

Among the experimentally tested particles, the used 200-nm MP were about the optimal size for this assay as smaller 50-nm MP decreased the magnetic signals while larger 950-nm particles exhibited poorer migration via the membrane (Fig. S4).

3.4. Correlation with ELISA

To verify the obtained results, we carried out a correlation analysis between the DRIM platform and a standard sandwich ELISA as a reference method using the same antibodies (Fig. 6). In these experiments, PSA was detected in 5 sets of clinical serum samples, one set per each of the following PSA diagnostics zones: Zero female control (0 ng/ml), Normal Level (< 1.5 ng/ml), Early-Warning Zone (1.5–4 ng/ml), Grey Zone (4–10 ng/ml), High Level (> 10 ng/ml).

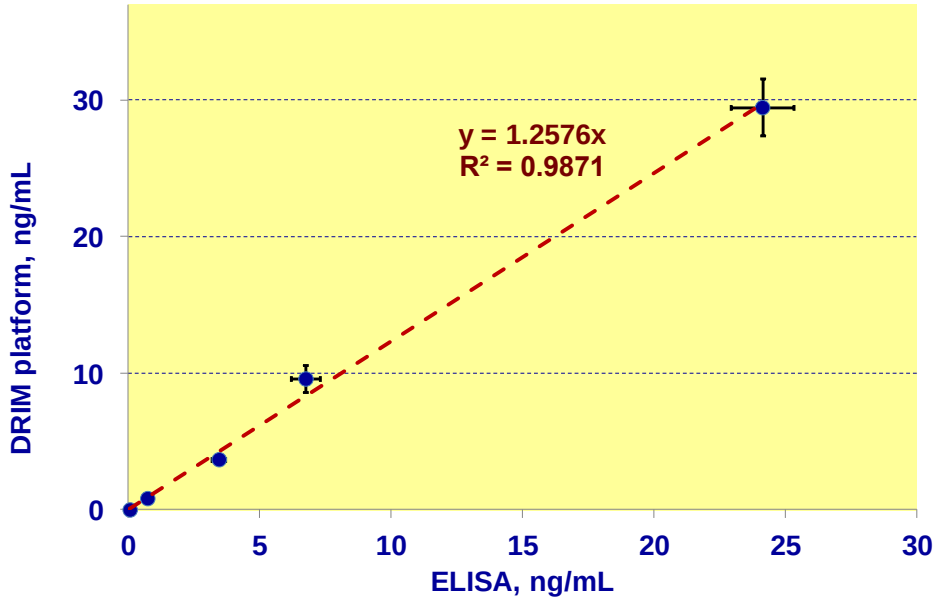

Fig. 6. Correlation between the results obtained with the DRIM platform and the reference method (ELISA)

The results obtained with both test systems in clinical serum samples showed high correlation (R^2 = 0.988), but the LOD for the DRIM platform was in 4 times better than that demonstrated with the standard ELISA using the same antibodies. The reliable reproducibility of CV < 10 % for PSA concentrations in clinical serum samples was achieved in experiments using 5 strips from different production batches. The slightly smaller concentration exhibited by ELISA may be due to different standards of antigen (see Supplementary material). The proposed platform provides not only better PSA detection in blood serum as compared to ELISA, it is also much simpler and faster. Importantly, dry chemistry provides long shelf-life of the diagnostic test strips and ease of use due to avoiding of external fluidics.

4. Discussion

4.1. The DRIM platform features

The DRIM platform features a very attractive LOD (≈ 740 fM) and high dose-response sensitivity (Fig. 5) with the signal growth by k_{log} = 3 times per an order of concentration within the dynamic range of 3.5 orders. One of the factors that elevate the dose-response sensitivity of the platform is the enhanced immunoactive surface area of the membrane, which is much larger than the sensing areas of typical flat 2D biochips. At the same time, the membrane has smaller reaction area in comparison with porous 3D filters (Orlov et al., 2013) and its 260-μm size (the thickest commercially available IC nitrocellulose) is still much smaller than the other geometric dimensions of the strip. Probably this is why those features of the developed assay, which relate to the MP size and reaction surface area, could formally be attributed to "2.5D assay" as they are in between the analytical characteristics of the assays on the flat 2D chips and on 3D filter structures. The absence of external fluidics and dry-reagent consumables in the DRIM platform are advantageous for many on-site applications.

4.2. Assay peculiarities due to the detection method

The used MPQ reader offers extremely high sensitivity and linearity. The detection from 60 zeptomoles of the 200-nm magnetic beads and very wide linear dynamic range is also an advantageous feature of the reader. To the best of the authors' knowledge, it has been the only

method that provides linear signal change by 7 orders against 7-order change in MP mass. This allowed MP recording not only on the TL and CL but also on various thick conjugation and absorption pads.
Importantly, the detectible magnetic signal is virtually independent of the strip position within the sensing area due to the chosen topology of the coils. Hence, the MP almost equally contribute to the signal regardless of how deeply in the membrane they are located. In fact, the MPQ device represents a high-accuracy selective magnetic "weighs", which detects in real time the mass of iron oxide nanocrystals within the sensing area and ignores other materials, including the MP beyond the area.
This feature offers an advantage over most optical and some other magnetometric methods, which practically do not take into account signals from the objects located deep from the surface. For example, optical methods for registration of traditional gold nanoparticles can detect only the labels localized not deeper than 10 μm from the surface. The information about the particles inside the rest 250 μm of the membrane thickness (about 96% of the total amount of labels), which could contribute to the useful signal, is lost. Besides, precise quantitative results are hardly obtainable with the optical methods at high concentrations (when the TL intensity is high) due to exponential Bouguer-Lambert-Beer law. In contrast, the DRIM platform registers all the magnetic nanolabels. Secondly, due to the high linearity of MPQ in the record wide dynamic range, the DRIM platform provides small errors not only near the detection limit but also when as large MP amounts as 7 orders of the minimal detectible value are present in the registration zone. It is these features that ensure sensitive and precise magnetic detection, as well as the advantageous combination of attractive LOD and wide dynamic range of the developed biosensor platform.

4.3. New tool for optimization and quality control of the test strips by MP mapping

The ability to register the particles over the entire depth of the strip and the wide dynamic range opens an opportunity previously inaccessible with other known methods, namely: real-time MP mapping along the strip while migration and bindings. We have thereby proposed the first tool for quantification of the nanolabel distribution during a biochemical reaction while strip displacement through the measuring coil (Fig. 5A). This tool may be used for optimization of assay parameters while development of IC strips and offers a basis for totally new approaches to quality control of the strips. For quickness, it is sufficient to record the signals from the TL and CL during the assay. In this research, while selection and optimization of the assay parameters, we took into account not only the signal from the TL but also the fraction of MP bound at each zone. For example, if the strip components did not fit perfectly to each other or to backing, the reader quantitatively counted the quantity of MP stuck on such imperfections. It may also be useful to evaluate the test efficiency after long storage by estimating the portion of the particles that participate in migration, which may decrease upon partial loss of stability due to improper storage, etc.
Another realized means for control of the test strip quality is monitoring the total MP mass in the test, which can be determined as the square under the curve of the MP distribution along the test strip. This parameter is independent of the antigen concentration and should be constant in every production batch of the tests. This characteristic can be used for device self-calibration and for control of irregularities in production technology, e.g., in the case of unexpected decrease in the MP-Ab conjugate amount to be deposited onto the strip.
It should be noted that one of the major reasons that impedes wide application of lateral flow IC tests in quantitative measurements is disbelief in their accuracy, in particular, due to non-existent control of the assay parameters. In fact, the only characteristic controllable by a user is visibility of the control line. The developed approach, in our opinion, will make the lateral

flow IC format controllable at every assay stage from development and optimization to measuring and interpretation of the assay results.

4.4. Perspectives of the DRIM platform

The developed assay is feasible for PSA detection in the clinically relevant concentration range even when the most highly sensitive registration is required, e.g., in serum of patients after radical prostatectomy, X-ray or gland therapy. The analytical characteristics similar to those demonstrated here will be achievable for a variety of proteomic biomarkers subject to the dissociation constant ranges for the specific monoclonal antibody-biomarker pairs. Therefore, along with the clinical relevance of the proposed method for PSA detection, the proposed approach may become a universal platform for rapid, highly sensitive and convenient detection of a wide range of biomarkers.

5. Conclusions

The developed dry-reagent immunomagnetic platform offers the advantages of rapid biosensing using ready-to-use kits for undiluted human serum. The platform performance was demonstrated by quantitative measurement of concentration of a model disease marker of prostate-specific antigen. An attractive combination of a medically relevant LOD (25 pg/ml or 740 fM), high dose-response sensitivity (the signal increases 3-fold per every order of concentration) within 3.5 orders dynamic range and high reproducibility (CV <10%) allows analysis of both low and high antigen concentrations. The achieved assay characteristics are superior to ELISA with using the same immunoreagents. The DRIM platform employs relatively small 200-nm magnetic nanolabels counted from entire volume of 260-μm IC membrane by the highly sensitive MPQ reader capable of detecting 60 zeptomoles of the nanolabels with the linear dynamic range of more than 7 orders of magnitude. Besides, the platform offers a new tool for control of immunochromatographic lateral flow tests by providing quantitative distribution of the MP along all the test strip components, not merely of the nitrocellulose membrane. This tool can be used for quick and convenient optimization of IC test systems, for thorough control of assay parameters and for enhancement of assay accuracy.

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Taton, K., Johnson, D., Guire, P., Lange, E., Tondra, M., 2009. J. Magn. Magn. Mater. 321(10), 1679-1682.Portable reader detects 60 zeptomoles of magnetic labels in 7-order linear dynamic range
Magnetic nanolabels are quantified from the entire volume of all lateral flow components
Platform offers quantitative on-site measurements of low and high analyte concentrations

Limit of detection of 25 pg/ml for PSA in human serum is achieved for 30-min test
Steep calibration curve is shown: 3-fold signal growth per order of concentration