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# Biosensor for total antinuclear antibody determination at the point-of-care

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ABSTRACT

Antinuclear antibodies (ANA) are important in diagnosis and follow-up of patients with autoimmune conditions. The current increase in ANA requests is driven by broadening the use of ANA from a test for lupus to a test for diverse autoimmune diseases, but the standard method is protracted, cumbersome and prone to error. We describe an electrochemical method for quantifying total ANA for use as a point-of-care diagnostic aid. In this technology the target autoantigens are derived from a protein/nucleoprotein mixture prepared from an inexpensive source and adsorbed to a porous membrane with high protein binding capacity. Serum is slowly drawn through the membrane comprising the high density autoantigen mixture to induce rapid binding of patient autoantibodies. After rinsing, peroxidase-conjugated anti-IgG is drawn through the membrane followed by rinsing, insertion of an electrode assembly, and addition of the enzyme substrate. Substrate peroxidation is measured by microamperage-level current accompanying electrochemical reduction of the intermediate product. Values are comparable to a standard ANA test but require a total processing time of ~20 min. This method has the promise to greatly expand ANA testing in clinical settings for initial patient assessment of autoimmune disease.



## 1. Introduction

Detection of ANA in blood is widely used as an initial test to support the diagnosis of an autoimmune connective tissue disease (Agmon-Levin et al., 2014; Meroni and Schur, 2010; Wiik, 2005) although it is sometimes positive in infectious, neurologic, liver, or neoplastic disease (Damoiseaux et al., 2015). However, the standard ANA test requires specialized laboratory equipment and expertise and is generally performed in a licensed clinical laboratory, which must absorb the substantial set-up and operational costs. The requirement for transport to the testing lab, blood processing, test execution, and communication of results creates a cumbersome, time-consuming, error prone and expensive process that detract from its diagnostic value.

Point-of-care (POC) testing has the potential to overcome these problems and is showing increasing use in emergency settings and the operating theater (Price, 2001). Biosensor technology offers the promise to streamline laboratory testing, thereby improving productivity of health care systems by minimizing costs, time, and errors. If available, reliable POC testing for ANA could improve diagnostics in primary, urgent/emergency, and remote care clinics and enhance medical intervention for patients (Konstantinov et al., 2013).

We describe an electrochemical (EC) biosensor for quantitation of ANA with sensitivity and specificity comparable to that of the gold standard ANA test (Agmon-Levin et al., 2014) performed in the clinical laboratory. This device for measuring total ANA in fresh blood has a simple, economical, and flexible design, uses inexpensive disposable materials and reagents, and produces a relatively rapid readout suitable for routine patient assessment for underlying autoimmune disease.

## 2. Materials and methods

### 2.1. Human serum samples

Blood was obtained from donors under auspices of a human subjects protocol approved by the Institutional Review Board. After clotting, serum was removed by centrifugation and stored at 5 °C in the presence of 0.05% sodium azide for < 6 weeks prior to use. Most of the patients were diagnosed at the University of New Mexico Health Sciences Center Division of Rheumatology with a systemic autoimmune rheumatic disease based on accepted criteria. ANA titer was determined by indirect immunofluorescence

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by TriCore Reference Laboratories, Albuquerque, NM, USA using 2-fold serial dilutions beginning at 1:40 to a maximum of 1:2560. Donors of normal blood were anonymous employees of the university. All samples were assigned number codes and used in the biosensor without knowledge of clinical laboratory information.

### 2.2. Autoantigens preparation

Rabbit thymus acetone powder (RTP-1000, Immunovision) was suspended in cold phosphate buffered saline (PBS, 0.14 M NaCl, 0.01 M Na phosphate, pH 7.4 with 0.01% thimerosol) at a ratio of 2 g per 30 ml (67 mg/ml), and 5 mg phenylmethylsulfonyl fluoride (Sigma-Aldrich) was added. The mixture was stirred at moderate rate overnight at 5 °C. After centrifugation at 3000 RPM for 10 min, the supernatant was re-centrifuged at 10,000 RPM for 60 min This slightly turbid rabbit thymus extract (RTE) had a protein content of ~4.0 mg/ml (BCA assay, Pierce Biotechnology), and was stored in 1.0 ml aliquots at −70 °C. Chromatin (chr), which is the natural DNA-histone macromolecular complex present in the nucleus, was purified from calf thymus (Pel-Freez) and stripped of histone H1 as previously described (Burlingame and Rubin, 1990) to maintain solubility. The concentration of its DNA component was determined by absorbance at 260 mμ based on E=25 for 1 mg/ml, and protein content was determined by the BCA assay. Protein/DNA ratio of the chromatin preparations was ~1.27. It was stored in liquid form in 50% glycerol at −20 °C at a concentration of ~0.3 mg DNA/ml.

### 2.3. Autoantigen-coated membrane for immunosensor

Polyvinylidene fluoride (PVDF, Pall Life Sciences) 1.0 cm × 8.25 cm membrane strips were hydrated as previously described (Rubin et al., 2014). In the standard coating protocol, membrane strips were submerged in 8.0 ml of antigen solution consisting of 4.2 mg RTE+0.17 mg chromatin ("RTE+chr") diluted in PBS (RTE/chromatin ratio=25). After continuous tube inversion for 5 min, the membrane was stored overnight at 5 °C, rinsed with PBS, and post-coated with 0.1% bovine serum albumin (BSA, Sigma)+1.0% polyoxyethylene sorbitan monolaurate (Tween-20, stabilized and peroxide/aldehyde-free, Sigma) in PBS for 2 h at room temperature. Antigen-coated membranes were either used the same day or stored at 5 °C in PBS containing 0.005% NP-40 (Sigma-Aldrich) 'wash solution'.

For protein titration studies hydrated duplicate 0.75 cm squares of PVDF membranes were placed in 0.55 ml RTE+chr solutions of increasing concentration and processed as described above except for omission of the BSA component of the post-coating solution. Final wash solution was replaced with 0.1 ml PBS and membrane-bound protein determined by the BCA assay. For comparison to antigenicity, increasing RTE+chr concentrations were incubated with PVDF strips, processed as usual, and used in the immunoassay described below.

### 2.4. Amperometric immunoassay

A manifold machined into 8 independently accessible 0.8 cm diameter chambers exposing 0.5 $cm^2$ of the underlying membrane was used (Rubin et al., 2014). All steps are performed at room temperature. Two-tenths ml of human sera (diluted 1:50 in 'serum diluent' containing 2.5 mg/ml bovine gamma globulin (Calbiochem EMD Biosciences)+3.5 mg/ml BSA in wash solution) were added to each chamber and simultaneously drawn through the antigen-coated membrane over the course of 2.5 min at 0.08 ml/min using a weak vacuum. Each well was then sequentially rinsed with 0.1 ml and then 0.4 ml wash solution at 0.7 ml/min. Then 0.2 ml peroxidase-conjugated rabbit anti-human IgG (Southern Biotech) diluted ~1:10,000 in serum diluent was drawn through the membrane at 0.2 ml/min followed by 3 rinses with 0.1 ml, 0.4 ml and 0.4 ml wash solution at 0.7 ml/min. After manually inserting the electrode array (Alderon Biosciences), comprising 8 sets of screen-printed electrodes each consisting of a carbon graphite working electrode surrounded by a counter electrode and a silver chloride reference electrode, 0.15 ml peroxidase substrate solution consisting of commercially-stabilized hydrogen peroxide ($H_2O_2$)+2 mM 3,3′,5,5′ tetramethylbenzidine ('TMB liquid substrate system', Sigma) was added to each well. Product accumulation over time was measured by intermittent pulse amperometry in which TMB charge-transfer complex (Josephy et al., 1982) undergoes electrochemical reduction at the working electrode (set at −100 mV vs. the reference electrode) (Rubin et al., 2014). An amperometric reader (Alderon Biosciences) recorded current between the working and counter electrode at 2 min intervals using 20, 5 ms voltage pulses during 4 s with results reported as the average of the last 15 pulses as previously described (Rubin et al., 2014). Thirteen min was the standard assay endpoint. Electrode uniformity was evaluated using TMB charge-transfer product freshly-prepared by incubating TMB substrate for ~4 min in membrane coated with peroxidase anti-human IgG/human IgG until 10X diluted product reached ~2.0 OD at 370 mμ.

### 2.5. Composition analysis of preparations by Western blot (WB)

RTE and H1-stripped chromatin were heated in 'sample buffer' and separated at room temperature (RT) under reducing conditions by sodium dodecyl sulfate 15% polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (Konstantinov et al., 1996). For DFS-70/LEDGF analysis 62 μg RTE, 4 μg H1-stripped chr and 17 μg nuclear extract of HeLa cells (Active Motif) were subjected to SDS-PAGE. After electrotransfer to PVDF membrane for 1 h at 100 V and 4 °C, proteins were visualized by staining with Ponceau S. All subsequent steps were performed at RT, and blots were washed 3X for 10 min between steps with PBS containing 0.05% Tween-20 (PBST). Blots were blocked for 1 h with 5% non-fat dried milk in PBST and sequentially incubated for 1 h with a collection of reference human autoimmune sera diluted 100X (Konstantinov et al., 1996) followed by 2000X diluted horseradish peroxidase-conjugated rabbit anti-human IgG (Southern Biotech) in blocking buffer. Antibody specificities of prototype sera were confirmed by WB and by indirect immunofluorescence microscopy at 1:100 dilution using HEp2 cells (Inova Diagnostics) and visualized with an Olympus IX70 microscope and digital camera. Prestained protein molecular markers (Fisher) were used to calibrate the immunoblots. Bound antibodies were detected by chemiluminescence (Clarity substrate, BioRad) according to the manufacturer's instructions.

### 2.6. ANA detection by enzyme-linked immunosorbent assay (ELISA)

ELISA for autoantibody reactivity to chromatin and/or RTE was employed as previously described (Burlingame and Rubin, 1990) except that antigen coating of RTE+chr wells was done by a sequential addition protocol in which RTE at 20 μg/ml was added 3 h after incubating wells with chromatin at 0.8 μg/ml (RTE/chromatin ratio=25). Post-coating wells with RTE after chromatin had no effect on detection of anti-chromatin antibody (using anti-RTE-negative sera, n=6, data not shown) but resulted in an average 30% reduction in anti-RTE reactivity compared to RTE alone (using anti-chromatin-negative sera, n=5, data not shown) presumably due to chromatin-mediated loss of RTE adsorption sites on the polystyrene wells.

## 3. Results

### 3.1. Performance of the electrochemical biosensor for ANA

Blood samples without anti-coagulant were obtained from patients undergoing assessment or treatment at the university outpatient rheumatology clinic. One tube was sent to the hospital clinical laboratory for ANA determination by indirect immunofluorescence (IF) and another tube was processed for serum collection and storage. Fig. 1 compares the two-fold incremental IF ANA titers with the continuous readout using the ANA biosensor. Despite that samples with the same ANA titer were resolved into quantitatively distinct reactivities by the biosensor, there was a good correlation between the two methods.

After addition of the peroxidase substrate, samples with moderate ANA activity displayed detectable signals by ~5 min in the biosensor, followed by a curvilinear increase that was essentially parallel for all samples (Supplement S1). By ~10 min the EC signal began to plateau due to substrate depletion and product inhibition of peroxidase activity (Rodriguez-Lopez et al., 1997). Samples from ANA negative, normal subjects showed no reactivity above that of the 'no serum' control, and there was negligible reactivity of any of the samples on membranes devoid of antigen. Total time for performing the sample manipulations was ~7 min plus 7–13 min for product development for a total elapsed time from addition of serum to final readout of 20 min when using the standard 13 min assay endpoint.

### 3.2. Basis for superior sensitivity of the ANA biosensor

Polyvinylidene fluoride (PVDF) membrane has remarkably high protein-binding capacity, just beginning to approach saturation at >600 μg/ml (Fig. 2). Approximately 50% of a 400 μg/ml RTE solution was initially adsorbed to 0.75 $cm^2$ membrane, although only ~30% of this material remained after post-coating with detergent (data not shown), leaving 70–80 μg of stably bound protein per square cm. As can be seen in Fig. 2, autoantibody reactivity in the biosensor increased proportionally to the amount of protein bound to the membrane. To balance ample sensor sensitivity and reagent conservation, our standard membrane coating with RTE+chromatin (chr) was 500 μg/ml, producing 35 μg RTE+chr adsorbed to the 0.5 $cm^2$ of membrane reaction surface.

While antibody to chromatin, to RTE and to a mixture of RTE+chr was readily detected in the EC biosensor, only anti-

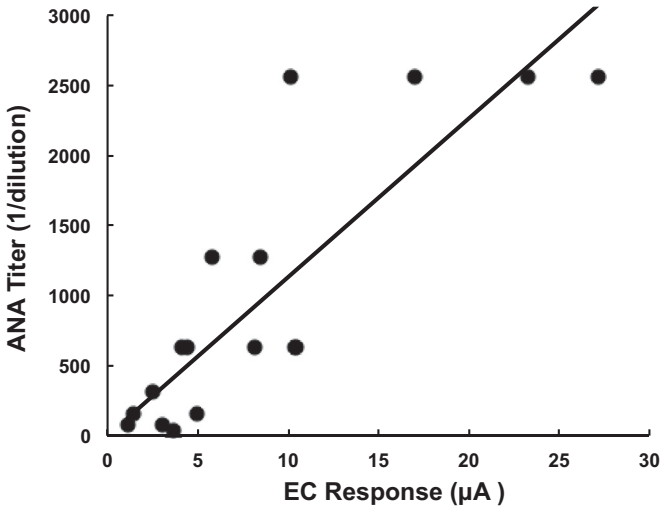


**Fig. 1.** Correlation between ANA activity determined by the EC biosensor vs. by a clinical laboratory (r=0.85).

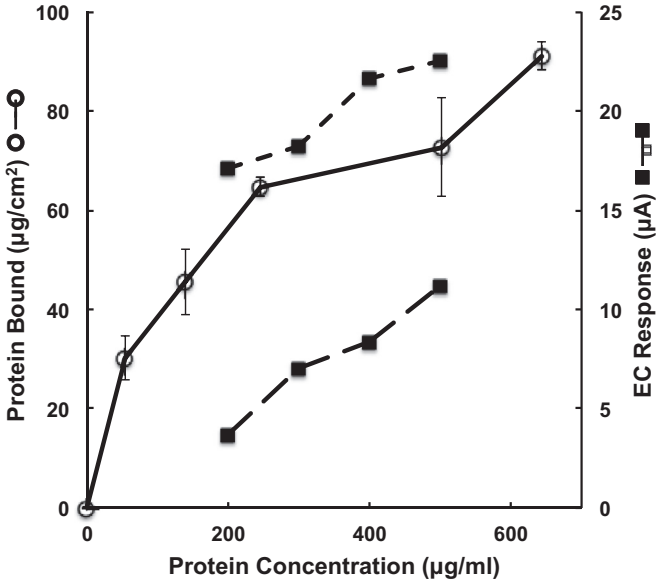


**Fig. 2.** Effect of concentration of RTE+chromatin on amount of protein stably bound to membranes after washing with 1% tween-20 (solid line and left axis), and on autoantibody activity of two ANA+ samples as measured by EC response (dashed lines and right axis). Error bars are S.D. at each input protein concentration; the average RSD over the entire range was 0.10.

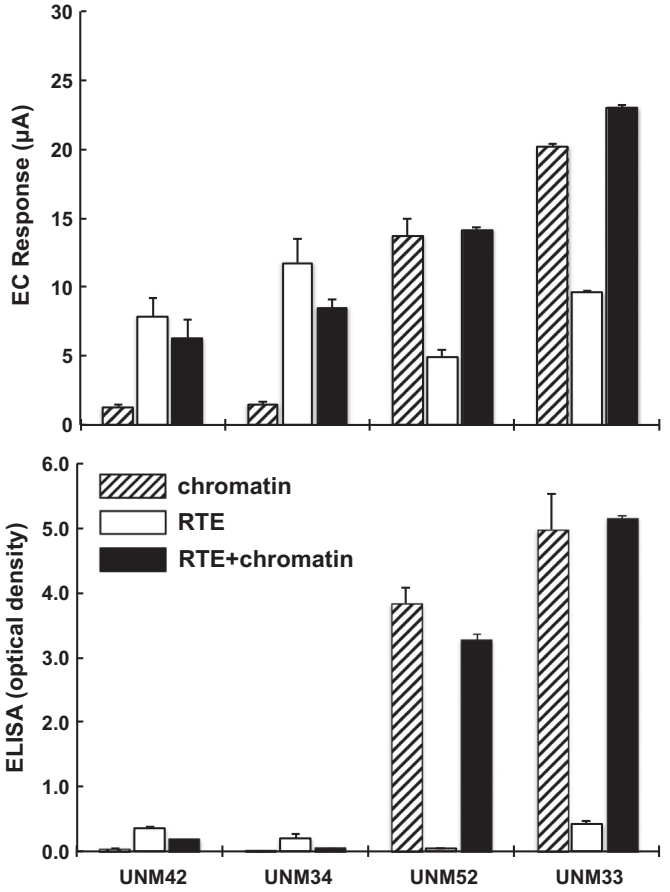


**Fig. 3.** Responsivity of EC sensor vs. ELISA to various antigen preparations. Reactivity of 4 ANA+ sera to chromatin, to RTE, or to RTE+chromatin bound to PVDF membrane and measured electrochemically (top) or bound to polystyrene and measured by ELISA (bottom). Error bars are S.D. The RSD in the EC response were 0.08, 0.11, and 0.08 for chromatin, RTE, and RTE+chromatin, respectively; for the ELISA, RSDs were 0.11, 0.11, 0.06, respectively.

chromatin antibodies produced a strong signal by ELISA (Fig. 3). In addition use of pre-mixed RTE+chr instead of a sequential coating protocol as described in M&M resulted in ~85% reduction in anti-chromatin activity by ELISA (data not shown), presumably because the 25-fold excess of RTE over chromatin largely blocked chromatin binding to the solid phase. In contrast, in the biosensor the mixture of RTE+chr lost only an average of ~30% antigenicity of either RTE or chromatin adsorbed separately to the membrane (Fig. 3). These results are attributed to the high protein binding capacity of PVDF membranes (Fig. 2), favoring detection of antibody reactivity to even minor proteins in a heterogeneous cell extract. Despite the relative insensitivity of ELISA for anti-RTE detection, it did show statistically significant correlation with the biosensor, especially when the RTE+chr mixture was used in both assays (Supplement S2). This correlation suggests that the non-covalent interactions stabilizing adsorption of autoantigens to polystyrene and PVDF membrane are similar and/or do not differentially affect epitope structure or accessibility.

### 3.3. Antigenic composition of the ANA biosensor

As expected from a crude extract of thymic tissue, the protein content of RTE is complex, ranging in molecular weight from < 10 kDa to > 200 kDa (Fig. 4A). H1-stripped chromatin consisted predominately of the 4 core histones. Major autoantigens that are characteristic targets of patients with autoimmune rheumatic diseases were evaluated by Western blot (WB) using a collection of reference sera expressing prototypic autoantibodies. As shown in Fig. 4B, RTE+chr reacted with antibodies to Sm, nuclear RNP, ribosomal RNP, SS-A, SS-B, Jo-1, Scl-70, and the core histones at their

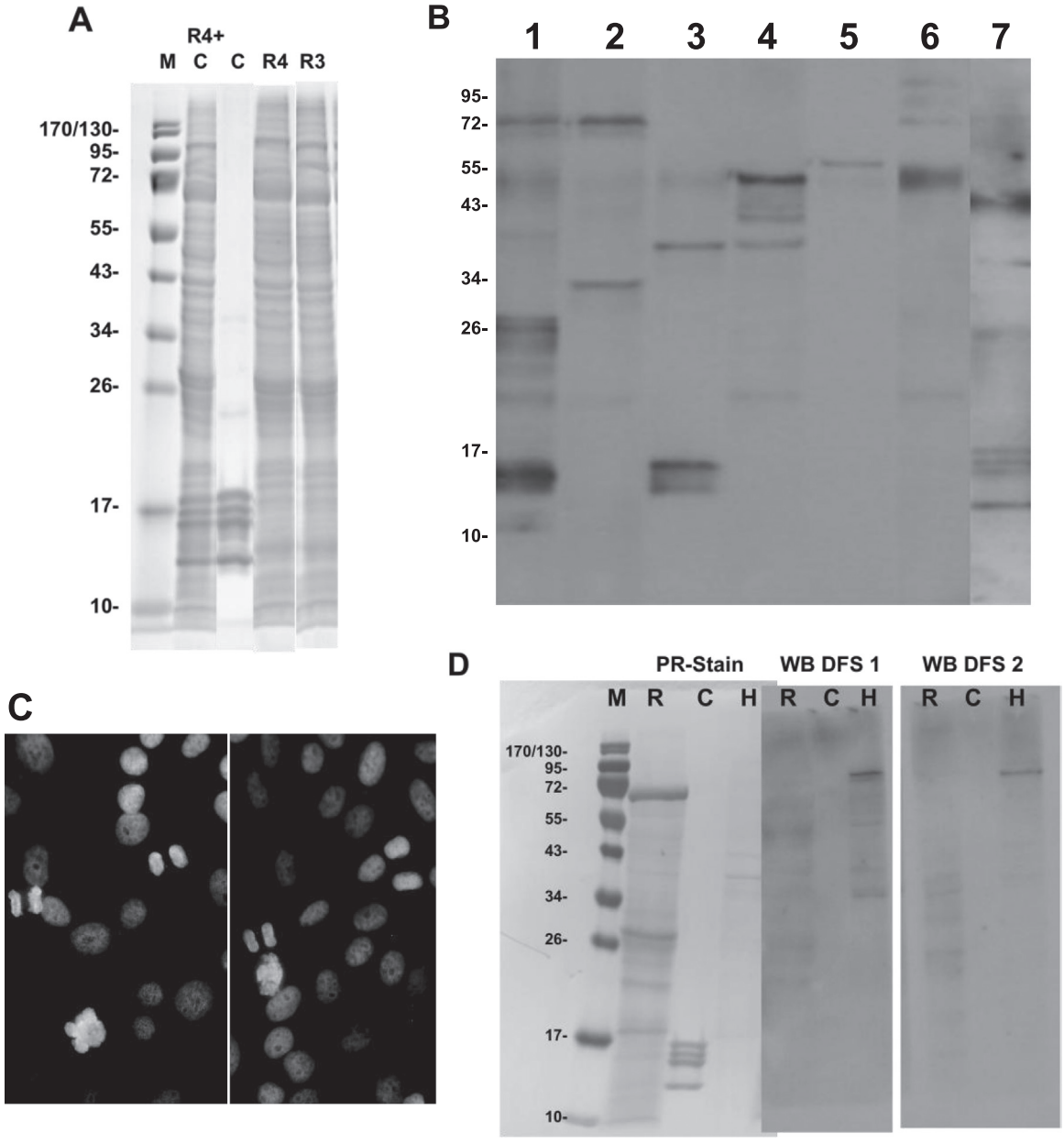


**Fig. 4.** Composition of the biosensor substrate. (A) Protein components separated by SDS-PAGE and stained with Coomassie blue. 'R3' and 'R4': two preparations of rabbit thymus extract; 'C': H1-stripped chromatin; M: molecular weight markers. (B) Autoantigenic properties of RTE+chromatin after SDS-PAGE and probed by WB with human reference sera. Reference antibodies are: 1: Sm, 2: nuclear RNP, 3: ribosomal RNP, 4: SS-A/SS-B, 5: Jo-1, 6: Scl-70, 7: histones. Molecular weight markers are shown on the left. (C) Immunofluorescence microscopy of 2 sera displaying the dense fine speckled staining pattern (D) LEDGF analysis by WB using the 2 DFS+ sea on substrates separated by SDS-PAGE. 'R': rabbit thymus extract; 'C': H1-stripped chromatin; 'H': extract of HeLa cell nuclei. Ponceau red (PR)-staining shows the protein composition of the blots after electrotransfer.

expected molecular weights (Peter, 1996).

A well-documented problem with the traditional immunofluorescence assay for ANA is the dense fine speckled (DFS) staining pattern due to antibodies to lens epithelium-derived growth factor p75 (LEDGF) (Mariz et al., 2011), which have little clinical significance and are generally considered false positive reactions (Mahler et al., 2012a). We used two sera with prototypic DFS staining (Fig. 4C) to probe for LEDGF in the antigen preparations used in the biosensor. These sera produced the expected reactivity with the 75 kDa LEDGF protein in WB using a nuclear extract of HeLa cells as a positive control but showed no reactivity to chromatin or to excess electrophoretically separated RTE (Fig. 4D).

### 3.4. Stability, reproducibility and durability of biosensor components

In its current form the biosensor consists of 8 sets of electrodes in a single assembly for simultaneous testing of 8 samples (Rubin et al., 2014). These individually addressable electrodes showed uniform EC responsivity to preformed tetramethylbenzidine (TMB) product, in which lack of background signal, responsivity to enzymatically-generated product and dynamic range carried over in a repeat assessment (Supplement S3). In the standard ANA determination, this was independent of the particular 8-set electrode assembly employed (Supplement S4). RTE preparations stored for at least 1 year showed similar antigenic activity as freshly prepared RTE (Supplement S5). Finally, stored [RTE+chr]-coated membrane displayed stable antigenic reactivity over the course of 6 months, with no significant change in sensitivity, inter-assay variance or background reactivity (Fig. 5).

## 4. Discussion

Autoantibodies are a hallmark of autoimmune diseases, and testing for total ANA has become a valuable tool at both primary care and subspecialty settings as a trigger for further clinical investigation. Standard ANA testing is performed by indirect immunofluorescence microscopy in centralized clinical laboratories by technicians trained in carrying out its multiple steps and in interpreting fluorescence microscopy images. Screening for individual autoantibodies in single or multiplexed arrays can also be undertaken using solid phase formats involving polystyrene microtiter plates (optical absorbance or fluorescence immunoassay), microscopic beads (fluorescently addressable laser bead fluorescence- or -chemiluminescence immunoassay), or adsorbent membranes (line immunoassay) (reviewed in Konstantinov et al., 2013; Agmon-Levin et al., 2014). In addition there are currently at least six commercial immunoassays for total ANA employing similar solid phase formats,[1] some of which have been recently evaluated and compared for diagnostic sensitivity and specificity in reference to the immunofluorescence assay using HEp2 cells (Kim, et al., 2012; Brito, et al., 2016). In these systems mixtures of relatively pure molecular targets are employed at considerable cost in order to approach the sensitivity of fluorescence microscopy for detecting autoantibodies to quantitatively minor cellular autoantigens. In addition, expensive analytical instrumentation as well as the relatively long turn-around time for obtaining results from centralized clinical laboratories limit the accessibility and usefulness of currently available tests for ANA. In the present report we describe a method and device for measuring total ANA in a single assay using a crude cell extract that is rapid, accurate, and inexpensive for both the disposable and non-disposable components, with potential for use at the clinical POC.

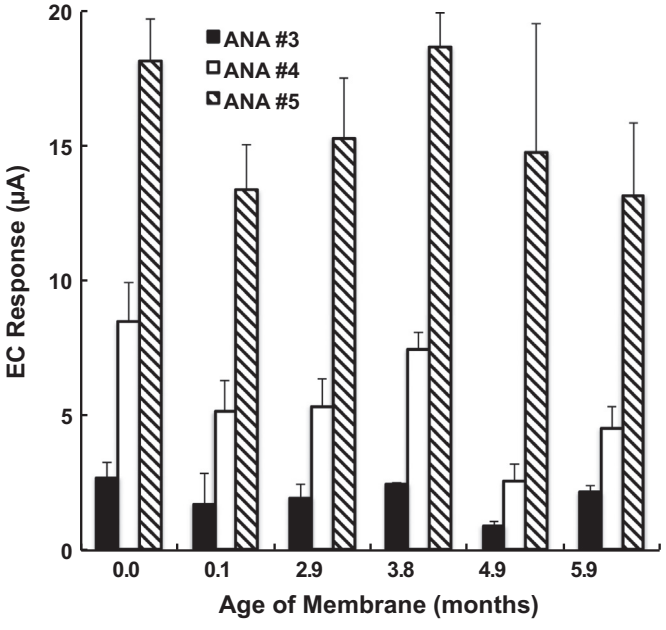


**Fig. 5.** Effect of storage time of membranes coated with RTE+chromatin on antigenicity as measured by EC responses to 3 sera. Error bars are S.D for serum sample replicates at each timepoint. The RSDs for samples ANA3, ANA4, and ANA5 over all the storage times were 0.3, 0.4, and 0.2, respectively.

Ability to detect autoantibody to a minor component of a crude cell extract required a high antigen density platform, amplification by secondary antibody linked to analyte formation mediated by conjugated enzyme, efficient and complete removal of irrelevant immunoglobulin facilitated by a porous immobilizing platform, and metered EC transduction of the analyte. Detection of specific autoantibody to such quantitatively minor antigens was not possible using two-dimensional antigen supports such as in ELISA format due to the limited protein adsorption properties of the polystyrene solid phase. Even when using a relatively pure antigenic target such as chromatin, the relatively low protein density on polystyrene necessitated a 2-h serum incubation time under agitation, compared to the 2.5 min that serum is drawn through the antigen-coated membrane of the biosensor.

As verified in the current work, thymus tissue is a rich source of nuclear antigens, accounting for its long-standing use in classical techniques such as Ouchterlony double immunodiffusion, counterimmunoelectrophoresis, and hemagglutination (Nakamura et al., 1985). An acetone powder of rabbit thymus extract (RTE) is commercially available and inexpensive. However, this material is largely devoid of DNA and nucleohistone due to the insolubility of chromatin in aqueous media. Because DNA, histones, and chromatin are important targets of autoantibodies (Burlingame et al., 1994), RTE was supplemented with in-house prepared chromatin solubilized by removal of histone H1. Mixing RTE with chromatin to 4% caused only a minor loss in autoantibody detection to the component antigens due to competition for membrane binding sites, but produced a more comprehensive target for the most common autoantibodies encountered in the rheumatic diseases. Although this mixture contained the major autoantibody targets, including Sm, snRNP, ribosomal RNP, SS-A, SS-B, Jo-1, Scl-70, histones, chromatin and dsDNA, some important autoantigens may not be present. While supplementing with purified and/or recombinant proteins for underrepresented autoantigens could

[1] ANAscreen (Orgentec Diagnostika GmbH, Mainz, Germany); Liaison® ANA Screen (DiaSorin, Saluggia, Italy); BioPlex™ 2200 (Bio-Rad, Hercules, CA, USA); Quanta Lite® ANA ELISA (INOVA Diagnostics Inc., San Diego, USA); Imtec-ANA Screen (Human Diagnostics GmbH, Wiesbaden, Germany); EL-ANAScr™ (TheraTest Labs, Lombard, USA).

increase the repertoire of detectable autoantibodies considering that the membrane adsorptive capacity is well below saturation, this would add to the cost of the disposable component of the biosensor.

A characteristic DFS staining pattern on HEp-2 cells is frequently observed in the standard ANA method (Mahler et al., 2012a). DFS staining is generally due to anti-LEDGF antibodies. DFS/anti-LEDGF has been reported positive in 9% (Mahler et al., 2012b), 11% (Watanabe et al., 2004), or 13% (Mariz et al., 2011) of individuals with no evidence of autoimmune disease or who develop autoimmune disease in the subsequent 4–5 years (Mariz et al., 2011) and is infrequent in individuals with systemic autoimmune diseases (Mahler et al., 2012a). Such false positive results detract from the usefulness of the ANA test for autoimmune disease (Mahler et al., 2012a). In our biosensor, LEDGF was undetectable by WB in the RTE or chromatin preparations, the latter presumably due to removal of loosely bound proteins along with histone H1 by the salt extraction procedure. As a result, the biosensor is expected to be more specific than the standard ANA for bona fide autoimmune disease, consistent with the failure of sera from normal individuals to produce any signal above background.

## 5. Conclusions

We describe a novel method of screening for total autoantibodies that displays lower risk for false positive results than the gold standard IF ANA test. The biosensor produces a digital readout on a linear scale comparable to quantitative IF or ELISA but superior in sensitivity to the latter due to the high protein-binding capacity of the membrane substrate. While the data in the present manuscript was obtained with a laboratory version of the device, we are in the process of re-engineering it into a portable prototype which would operate semi-automatically for the goal of implementing ANA testing at the clinical point-of-care. While such a device should be relatively inexpensive to manufacture, the non-disposable components may have to be rinsed or cleaned between use. Nevertheless, by virtue of its near real time data collection capability, the biosensor offers the prospect of testing patients at their initial visit when autoimmune disease is a diagnostic consideration.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.04.048.

## References

Agmon-Levin, N., Damoiseaux, J., Kallenberg, C., Sack, U., Witte, T., Herold, M., Bossuyt, X., Musset, L., Cervera, R., Plaza-Lopez, A., Dias, C., Sousa, M.J., Radice, A., Eriksson, C., Hultgren, O., Viander, M., Khamashta, M., Regenass, S., Andrade, L.E., Wiik, A., Tincani, A., Ronnelid, J., Bloch, D.B., Fritzler, M.J., Chan, E.K., Garcia-De La Torre, I., Konstantinov, K.N., Lahita, R., Wilson, M., Vainio, O., Fabien, N., Sinico, R.A., Meroni, P., Shoenfeld, Y., 2014. Ann. Rheum. Dis. 73, 17–23.
Brito, F.A., Santos, M.S., Ferreira, G.A., Pedrosa, W., Gradisse, J., Costa, L.C., Neves, S.P. F., 2016. Am. J. Clin. Pathol. 145, 323–331.
Burlingame, R.W., Boey, M.L., Starkebaum, G., Rubin, R.L., 1994. J. Clin. Invest. 94, 184–192.
Burlingame, R.W., Rubin, R.L., 1990. J. Immunol. Methods 134, 187–199.
Damoiseaux, J., Andrade, L.E., Fritzler, M.J., Shoenfeld, Y., 2015. Autoimmun. Rev. 14, 555–563.
Josephy, P.D., Eling, T., Mason, R.P., 1982. J. Biol. Chem. 257, 3669–3675.
Kim, K., Park, Y., Lee, E.Y., Kim, H.-S., 2012. Clin. Chim. Acta 413, 308–311.
Konstantinov, K., von Mikecz, A., Buchwald, D., Jones, J., Gerace, L., Tan, E.M., 1996. J. Clin. Invest. 98, 1888–1896.
Konstantinov, K.N., Tzamaloukas, A., Rubin, R.L., 2013. Auto Immun. Highlights 4, 55–61.
Mahler, M., Hanly, J.G., Fritzler, M.J., 2012a. Autoimmun. Rev. 11, 642–645.
Mahler, M., Parker, T., Peebles, C.L., Andrade, L.E., Swart, A., Carbone, Y., Ferguson, D. J., Villalta, D., Bizzaro, N., Hanly, J.G., Fritzler, M.J., 2012b. J. Rheumatol. 39, 2104–2110.
Mariz, H.A., Sato, E.I., Barbosa, S.H., Rodrigues, S.H., Dellavance, A., Andrade, L.E., 2011. Arthritis Rheumatol. 63, 191–200.
Meroni, P.L., Schur, P.H., 2010. Ann. Rheum. Dis. 69, 1420–1422.
Nakamura, R.M., Peebles, C., Rubin, R.L., Molden, D.P., Tan, E.M., 1985. Autoantibodies to Nuclear Antigens (ANA), 2nd ed. American Society of Clinical Pathologists Press, Chicago.
Peter, J., Schoenfeld, D., 1996. Autoantibodies. Elsevier Science B.V., Amsterdam.
Price, C.P., 2001. BMJ 322, 1285–1288.
Rodriguez-Lopez, J.N., Hernandez-Ruiz, J., Garcia-Canovas, F., Thorneley, R.N., Acosta, M., Arnao, M.B., 1997. J. Biol. Chem. 272, 5469–5476.
Rubin, R.L., Wall, D., Konstantinov, K.N., 2014. Biosens. Bioelectron. 51, 177–183.
Watanabe, A., Kodera, M., Sugiura, K., Usuda, T., Tan, E.M., Takasaki, Y., Tomita, Y., Muro, Y., 2004. Arthritis Rheumatol. 50, 892–900.
Wiik, A.S., 2005. Scand. J. Rheumatol. 34, 260–268.