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Desktop scanner replaces spectrophotometer for low cost and rapid ELISA technique

Pradip Nahar (pnahar@igib.res.in)
Nahar's Lab
Shahila Parween
Nahar's Lab

Method Article

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Abstract

In this protocol, we report spectrophotometer-free image-based miniaturized ELISA on an activated polypropylene microtest plate \(APPµTP) which is precise, reliable, rapid, and sensitive. Activated test zone in APPµTP binds a capture biomolecule through covalent linkage thereby, eliminating non-specific binding often prevalent in absorption based techniques. Efficacy of APPµTP is demonstrated by detecting human immunoglobulin G \(IgG), human immunoglobulin E \(IgE) and Aspergillus fumigatus antibody in patient's sera. Detection is done by taking the image of the assay solution by a desktop scanner and analyzing the color of the image. Human IgE quantification by color saturation in the image-based assay shows excellent correlation with absorbance-based assay. APPµTP can quantify an analyte as precisely as in microtiter plate and could be a good substitute for conventional immunoassay procedure widely used in clinical and research laboratories.

Introduction

Earlier, we have reduced the ELISA procedure time by doing it on a photoactivated polystyrene microtiter plate \(1). Just by activating the plate around 1.5–2 fold higher reading was obtained than the untreated plate when the assay was carried out at 37 degree celsius in around 8 h \(2). Performing ELISA in the wells of a photoactivated polystyrene microtiter plate and applying different unconventional incubation procedures we have further shortend ELISA timing with the retention of sensitivity and specificity \(3-5). Thus, ELISA steps at elevated temperature \(Heat- mediated ELISA or HELISA) were completed in less than 3 h \(3) where as ELISA steps by pressure incubation \(pressure-mediated ELISA or PELISA) was done in 60 minutes only \(4). In another breakthrough invention, ultrasound incubation \(soundwave - mediated ELISA or SELISA) has dramatically reduced the total time required for ELISA to 40 minutes \(5). In the present innovative approach we have carried out ELISA without using spectrophotometer or polystyrene microtiter plate \(6). The promising features of this assay are: \(i) it is image-based; hence no need of spectrophotometer, \(ii) miniaturized; hence requires less amount of reagents and analyte, \(iii) rapid \(iv) sensitive and \(v) specific.

Reagents

• 1-fluoro-2-nitro-4-azidobenzene \(FNAB, IUPAC nomenclature: 4-azido-1-fluoro-2-nitrobenzene) can be purchased from Apollo scientific ltd, UK \(cat no. 248-878-6). Alternatively, it can be made from 4-fluoro-3-nitroaniline by a simple diazotization reaction as described earlier \(1,7) CAUTION. FNAB is explosive and should be handled with care, especially when using large quantities. • Polystyren microtiter plates \(Greiner Labortechnik, Germany). • Anti-human IgG, \(Sigma Aldrich, cat. no. I3382) CAUTION Store all reagents at 2-8 degree celsius. If slight turbidity occurs upon prolonged storage clarify the solution by centrifugation before use. • Human IgG, \(Sigma Aldrich, cat. no. I4506). • Anti-human IgG-HRP conjugate, \(Sigma Aldrich, cat. no. A8419). • Rabbit IgG, \(Sigma Aldrich, cat. no. I4508) • Human IgE ELISA Quantitation Set \(Cat. no. E80-108, Bethyl laboratories, USA). CAUTION Store all reagents at 2-8 degree celsius. Do not freeze reagents. All reagents must be kept at room temperature \(20-25 degree celsius)

before use. Components supplied in the kit are: Affinity purified Goat anti-Human IgE Coating Antibody A80-108A, 1 ml at 1 mg/ml Human IgE Calibrator, RC80-108-6, 1.0 ml HRP Conjugated Goat anti-Human IgE Detection Antibody A80-108P, 0.1 ml at 1 mg/ml • Bovine serum albumin \(BSA), Sigma \(USA). CRITICAL BSA solution should be filtered prior to use to avoid microbial contamination. • o-phenylenediamine dihydrochloride \(OPD), \(Sigma Aldrich, USA cat. no. P1526). Store in cool place. Recommended storage temperature: -20 degree celsius; Keep container tightly closed. CAUTION Avoid contact with skin and eyes. Avoid formation of dust and aerosols. • Phosphate buffered saline \(PBS) was prepared by mixing 0.85% sodium chloride to 0.01 M phosphate buffer \(pH 7.2). To make 0.01 M phosphate buffer add 1.217 g disodium phosphate and 0.379 g monosodium phosphate to 500 ml of distilled water. Add distilled water to make the volume upto 1 liter. • Washing buffer was made by adding 0.1% Tween 20 to PBS. • Substrate -dye buffer was prepared by mixing 12 ml of citrate buffer \(0.025M citric acid and 0.05 M sodium phosphate dibasic , pH 5), 5 µl of hydrogen peroxide \(30% w/v), and 4 mg of o-phenylenediamine dihydrochloride. • Stop Solution- 5% sulphuric acid

Equipment

• Refrigerator \(LG Electronics, India) • UV Stratalinker 2400 \(Stratagene, USA) • Polystyrene microtiter plates \(Greiner Labortechnik, Germany) • Sonicator bath having a frequency of 40 KHz \(Elma Transonic Digitals, Germany) • ELISA reader \(Biorad iMark™ Microplate Reader, USA) • Desktop scanner \(HP photo smart C6388)

Procedure

1. Preparation of PPµTP PPµTP was made from locally purchased white polypropylene sheet. The sheet was cut into a strip having dimension of 9 cm in length and 3 cm in width. On the strip, array of test zones \(small cavities) were made by mildly hammering or pressing with a blunt end metal rod. Each cavity was separated by 0.5 cm. Cavity of the PPµTP had an average diameter of 3.5 mm and a depth of around 1 mm. All the dimensions were measured by an electronic caliper. 2. Activation of PPµTP The wells of a PPµTP were activated by pouring FNAB \(0.0625 mg/2.5µl of methanol/cavity) solution to each well followed by slow evaporation of the solvent. The dry coated plate was activated by UV light for 20 min at a wavelength of 365 nm as described \(1). CRITICAL Fast evaporation may remove FNAB from the bottom of the cavity to the top. In such case activation may not occur at the bottom. 3. Detection of analyte Analyte \(human IgG, human IgE) was detected by immobilizing a capture molecule \(anti-human IgG, anti-human IgE,) onto the activated and untreated cavities of PPµTP, blocking, binding of analyte and binding of secondary antibody-conjugate by ultrasound waves in a sonicator bath operating at a frequency of 40 KHz and an output power of 120 W. The plates were washed after each step. Color development was carried out by adding 8µl of substrate- dye buffer and then stopped by adding 2µl of 5% sulphuric acid. 4. Image- based quantification The PPµTP was then scanned on a desktop scanner \(HP photo smart C6388) by placing the plate upside down to get the image. From Adobe Photoshop the mean value of each R, G and B of the scanned image was obtained which was then converted to HSB \

(Hue, Saturation, and Brightness) using freely available 'Macbeth color calculator' software. Image was then quantified as color saturation percentage.

Timing

1. Activation of polystyrene surface –approx. 25 minutes a. Coating of FNAB to polystyrene surface 5-10 minutes b. UV light exposure- 15 minutes c. Washing and drying - 5 minutes 2. Detection of Analyte - 40 minutes + washing time after each step a) Immobilization of capture molecule \(10 pg /10 µl 0.01 M carbonate buffer, pH 9.6/well) onto the photoactivated surface by ultrasound waves in a sonicator bath for 10 minutes at 37 degree celsius. b) Blocking \(10 µl/ well of 2% BSA) by ultrasound waves in a sonicator bath for 10 minutes at 37 degree celsius . c) Incubation of analyte \(10 pg /10 µl 0.01 M PBS, pH 7.2/well) by ultrasound waves in a sonicator bath for 10 minutes at 37 degree celsius. d) Incubation time of secondary antibody \(10 µl of 1:2000) dilution by ultrasound waves in a sonicator bath 10 minutes at 37 degree celsius.

Anticipated Results

It is image-based; hence no need of spectrophotometer • Miniaturized; hence requires less amount of reagents and analyte, • Rapid; although, 40 min-ultrasound ELISA is comparable with 18-h ELISA, the analyte on APPµTP can be detected even in 8 minutes. That means if first two steps are prefabricated, ELISA can be done in 4 min only \(Fig. 2). Being one of the fastest ELISA in the current scenario, the 4 min-ELISA can be used as a point of care \(POC) diagnosis. • Sensitive • Specific. • The results shows that analyte detection on APPµTP is akin to that of microtiter based assay with no non-specific. • There is negligible non-specific binding on APPµTP ELISA, either performed by long conventional incubation or rapid ultrasonic waves.

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Figures

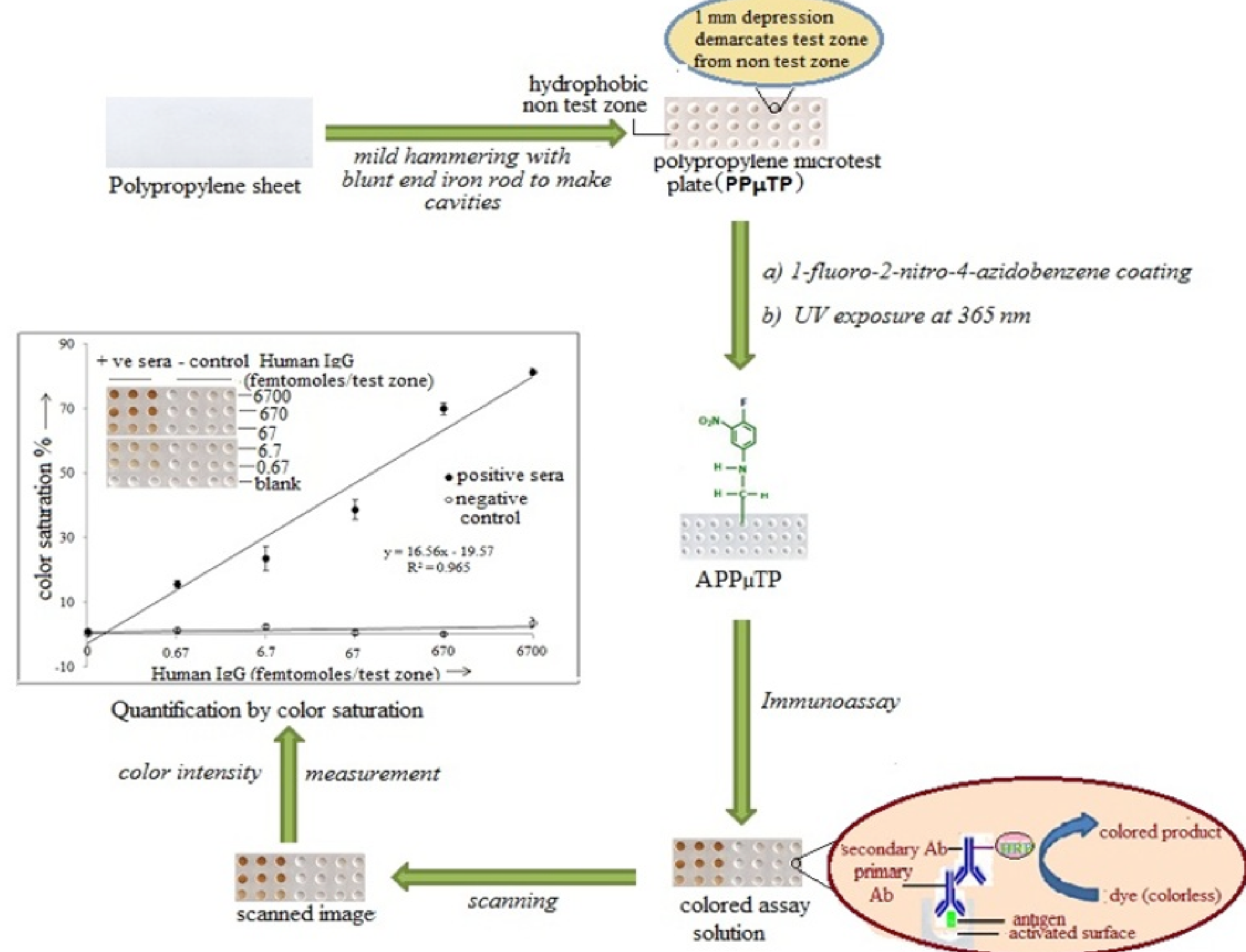

Figure 1

Schematic representation of image-based ELISA on APPμTP. Schematic representation of image-based ELISA on APPμTP. Test zones were activated by FNAB for covalent immobilization of a capture molecule. Color assay solution was scanned and the intensity of color was analysed as color saturation percentage.

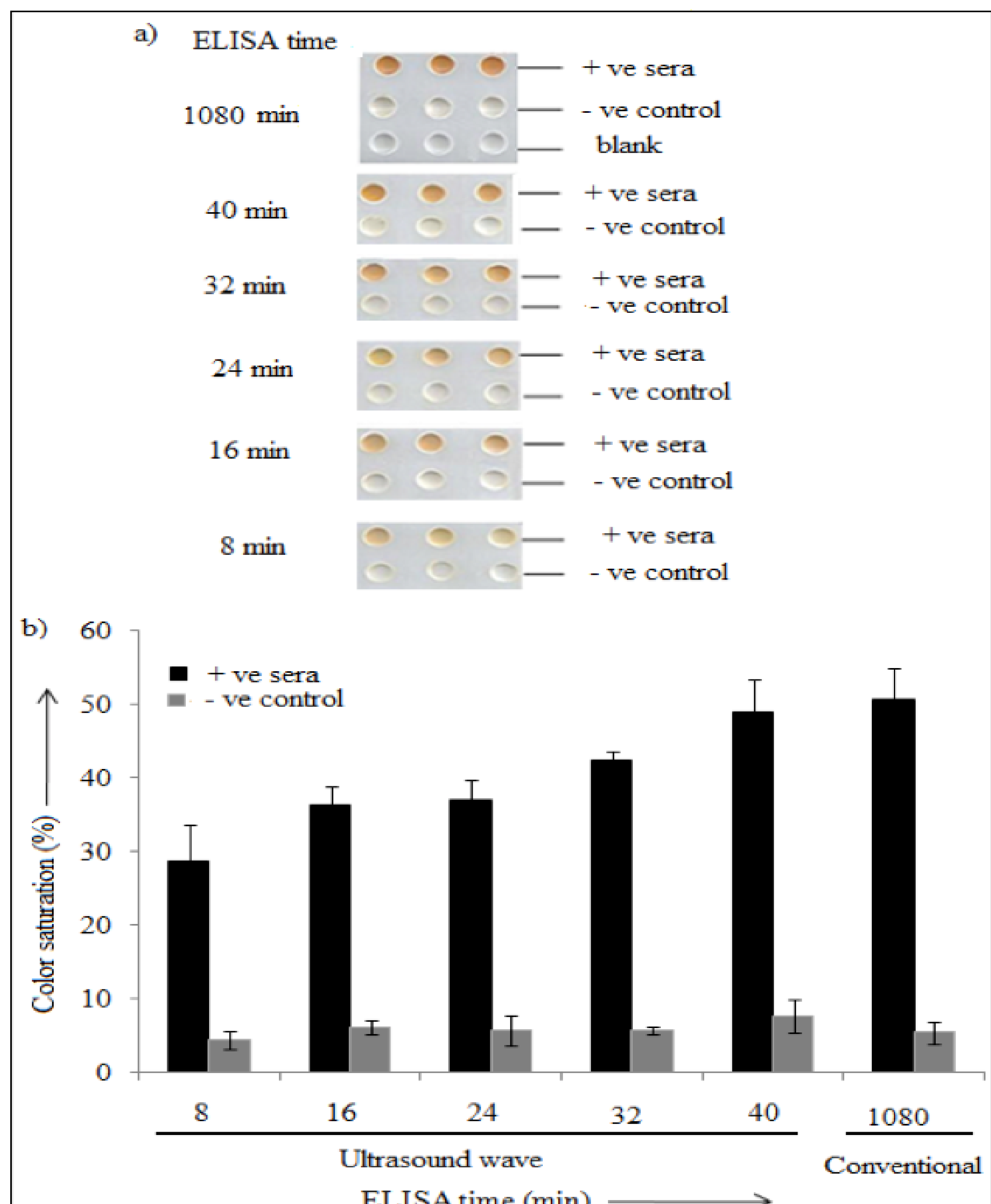

Figure 2

Detection of human IgG by rapid ELISA on APPμTP by ultrasound waves in different times. Detection of human IgG by rapid ELISA on APPμTP by ultrasound waves in different times. Detection of human IgG by conventional ELISA was carried out in 18 hours. (a) Scanned images of experiments. (b) Quantification of resultant colored assay solutions by color saturation.