Rapid enzyme-linked immunosorbent assay technique by ultrasound waves in a sonicator bath

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

Method Article

Keywords: Sonication, enzyme-linked, immunosorbent, assay, SELISA, Photoactivation, 1-Fluoro-2-nitro-4-azidobenzene, Ultrasound

Posted Date: December 12th, 2013

DOI: https://doi.org/10.1038/protex.2013.093

Abstract

This protocol describes a novel ultrasound-mediated ELISA procedure that can dramatically reduce the ELISA timing to 40 minutes without losing its specificity or sensitivity. Ultrasound-mediated ELISA was best achieved on an activated microtiter plate which was able to covalently bind antigen or antibody. Binding occured in 10 min when subjected to ultrasound waves in a sonicator bath, operating at an output power of 120 W. Blocking, antibody binding and secondary antibody–enzyme conjugate binding were also accomplished in 10 min each in the sonicator bath under similar conditions. Total IgE detection by 40 min-SELISA method gives similar absorbance value to that obtained by 20 h- conventional procedure. In fact any type of ELISA can be carried out by SELISA technique. SELISA method is sensitive and can detect lower concentration of analyte that could be significantly useful to confirm false negative cases. In short, SELISA procedure is rapid, sensitive, specific and reproducible; hence better option than conventional ELISA procedure.

Introduction

With the increasing burden of infectious diseases, it has become important to develop a rapid ELISA that could facilitate early diagnosis. Earlier, we have demonstrated that the solid phase prepared on a photoactivated polystyrene microtiter plate \(1) gave around 1.5–2 fold higher readings than the untreated surface when the assay was carried out at 37 degree celsius in around 8 h \(2). Further we have reduced the ELISA timing to less than 3 h by doing it at elevated temperature \(Heat- mediated ELISA or HELISA) onto the photoactivated plate \(3). We have also shown that pressure incubation \(pressure-mediated ELISA or PELISA) can further reduce ELISA timming to 1 h \(4). Herein, we report the use of ultrasound waves, an alternate source of energy, in reducing ELISA timing for antibody detection. The procedure has dramatically reduced the total time required for ELISA to 40 min with the retention of sensitivity and specificity \(5).

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 \(1,6) CAUTION. FNAB is explosive and should be handled with care, especially when using large quantities. We did not encounter any untoward incident while working with FNAB in last 12 years. • 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) • 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 \(Godrej, India) • UV Stratalinker 2400 \(Stratagene, USA) • Polystyrene microtiter plates \(Greiner Labortechnik, Germany) • ELISA reader \(Biorad iMark™ Microplate Reader, USA). • Sonicator bath having a frequency of 40 KHz \(Elma Transonic Digitals, Germany)

Procedure

1. Activation of polystyrene surface The wells of a polystyrene plate were activated by coating with FNAB followed by photoactivation by UV light for 15 min at a wavelength of 365 nm as described \(1). CRITICAL We have decreased UV exposure time for activation of microtiter plate from 20 minutes to 15 minutes without any significant difference in results. 2. Detection of analyte In activated and untreated polystyrene microtiter plates analyte \(human IgG, human IgE) was detected by immobilizing capture molecule \(anti-human IgG, anti-human IgE,) onto the activated and untreated polystyrene microtiter plates by ultrasound waves in a sonicator bath followed by blocking , binding of analyte and secondary antibody-conjugate by the same conditions. The plates were washed after each step. Color development was carried out by adding 100 µl of substrate- dye buffer and then stopped by adding 20 µl of 5% sulphuric acid. Absorbance was recorded at 490 nm.

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 \(0.125 µg/100 µl 0.01 M carbonate buffer, pH 9.6/well) onto the photoactivated surface- sonication at an output power of 120 W in a sonicator bath for 10 minutes at 37 degree celsius. b) Blocking \(200 µl/ well of 2% BSA) - sonication at an output power of 120 W in a sonicator bath for 10 minutes at 37 degree celsius . c) Incubation of analyte \(0.125 µg/100 µl 0.01 M PBS, pH 7.2/well) - sonication at an output power of 120 W in a sonicator bath for 10 minutes at 37 degree celsius. d) Incubation time of secondary antibody \(100 µl of 1:5000) dilution - sonication at an output power of 120 W in a sonicator bath for 10 minutes at 37 degree celsius.

Anticipated Results

• SELISA steps were carried out in a sonicator bath having a frequency of 40 KHz and an output power of 120 W. • Total time for covalent immobilization of goat anti-human IgG, blocking, human IgG binding and anti-human IgG peroxidase binding by ultrasound waves was 40 min. Time for each step in SELISA was 10 min only. • We have further compared SELISA with conventional ELISA and HELISA. Absorbance value obtained in SELISA was akin to that of conventional ELISA and HELISA. To find out whether ultrasound waves indeed accelerated ELISA, we also performed ELISA at 37 degree celsius but without applying sonication. Control experiment carried out without sonication showed very low absorbance values on both treated and untreated surface, thus confirming the role of ultrasound waves as an accelerator of ELISA \(Fig. 1)

References

1. \(a) Nahar P. Light-Induced Activation of an Inert Surface for Covalent Immobilization of a Protein Ligand, Community Contributed Protocol Exchange 26/11/2013 doi:10.1038/protex.2013.086. \(b) Nahar, P., Wali, N.M., Gandhi, R.P., 2001. Light-induced activation of an inert surface for covalent immobilization of protein ligand. Anal. Biochem. 294, 148. 2. \(a) Nahar P., Covalent immobilization of proteins onto photoactivated polystyrene microtiter plates for enzyme-linked immunosorbent assay procedures. Community Contributed Protocol Exchange 05/12/2013 doi:10.1038/protex.2013.090. \(b) Bora, U., Chugh, L., Nahar, P., 2002. Covalent immobilization of proteins onto photoactivated polystyrene microtiter plates for enzyme-linked immunosorbent assay procedures. J. Immunol. Methods 268, 171. 3. \(a) Nahar P., Enzyme-linked immunosorbent assay procedure at higher temperature enhances speed of the assay. Community Contributed Protocol Exchange 09/12/2013 doi:10.1038/protex.2013.091. \(b) Bora, U., Kannan, K. and Nahar, P. \(2004) Heat-mediated enzyme-linked immunosorbent assay \(HELISA) Procedure on a photoactivated surface. Journal of Immunol Methods. 293: 43-50. \(c) Nahar P and Bora U. Rapid Heat-Mediated Method for Enzyme Linked Immunosorbent Assay Procedure. European Patent \(EP) 1608971. \(d) Kumar S, Ghosh L, Kumar S, Ghosh B, Nahar P \(2007) A rapid method for detection of cell adhesion molecules \(CAMs) on human umbilical vein endothelial cells \(HUVECs). Talanta 73: 466–470. \(e) Sharma P, Gupta B, Basir S F, Das H R, Nahar P \(2008). Rapid and sensitive detection of autoantibody in rheumatoid arthritis patients by heat-mediated ELISA. Clinical Biochemistry 41: 97–102 4. \(a) Nahar P., A novel enzyme-linked immunosorbent assay procedure in one hour by pressure incubation . Community Contributed Protocol Exchange 09/12/2013 doi:10.1038/protex.2013.092. \(b) Kannoujia DK and Nahar P \(2009) Pressure: a novel tool for enzyme-linked immunosorbent assay procedure. BioTechniques 46: 468–472. \(c) Nahar Pradip and Kumar Dileep. A Novel Pressure -Induced Immunoassay Procedure. Patent pending. 5. \(a) Pragya Sharma, Pradip Nahar \(2009) Ultrasound wave-mediated enzyme-linked immunosorbent assay technique. Analytica Chimica Acta 650 241–246. \(b) Faster ELISA test- Research highlight, Nature India \(2009), doi:10.1038/nindia.2009.289. \(c) Nahar P and Sharma P. An Enzyme Linked Immunosorbent Assay Procedure by sonication. Patent pending. 6. Naqvi, A., Nahar, P., 2004. Photochemical immobilization of proteins on microwave-synthesized photoreactive polymers. Anal. Biochem. 327, 68.

Figures

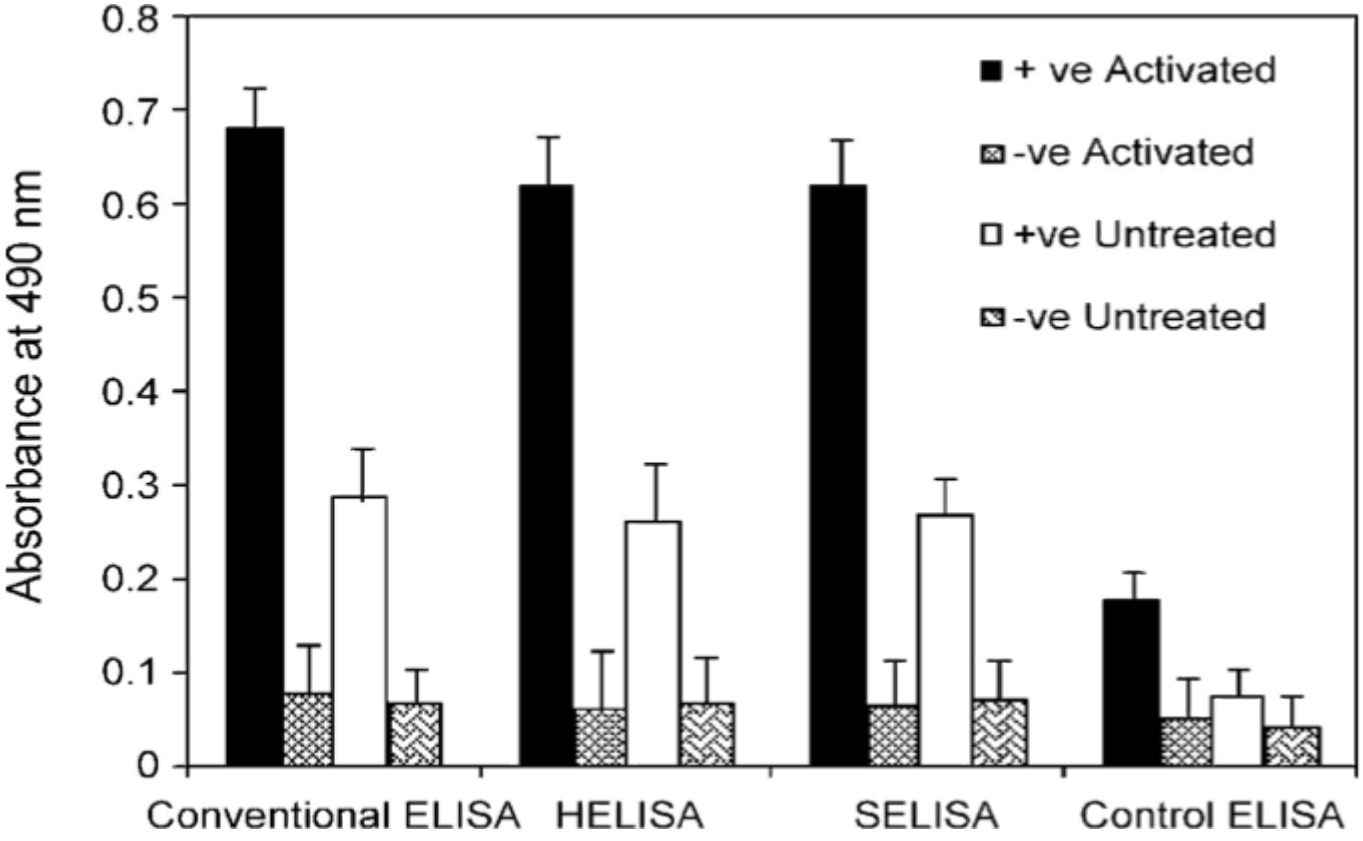

Figure 1

Comparison between 20 h-conventional ELISA, 2 h 50 min-HELISA, 40 min-SELISA and 40 min-control ELISA. Comparison between 20 h-conventional ELISA, 2 h 50 min-HELISA, 40 min-SELISA and 40 min-control ELISA. Control ELISA was carried out in the same temperature and time as in SELISA but without sonication.