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A gold standard method for the evaluation of antibody-based materials functionality: Approach to forced degradation studies

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ABSTRACT

The scope of this paper is to present a gold standard method to evaluate functional activity of antibody (Ab)-based materials during the different phases of their development, after their exposure to forced degradations or even during routine quality control. Ab-based materials play a central role in the development of diagnostic devices, for example, for screening or therapeutic target characterization, in formulation development, and in novel micro(nano)technology approaches to develop immunosensors useful for the analysis of trace substances in pharmaceutical and food industries, clinical and environmental fields. A very important aspect in diagnostic device development is the construction of its biofunctional surfaces. These Ab surfaces require biocompatibility, homogeneity, stability, specificity and functionality.

Thus, this work describes the validation and applications of a unique ligand binding assay to directly perform the quantitative measurement of functional Ab binding sites immobilized on the solid surfaces. The method called Antibody Anti-HorseRadish Peroxidase (A2HRP) method, uses a covalently coated anti-HRP antibody (anti-HRP Ab) and does not need for a secondary Ab during the detection step. The A2HRP method was validated and gave reliable results over a wide range of absorbance values. Analyzed validation criteria were fulfilled as requested by the food and drug administration (FDA) and European Medicines Agency (EMA) guidance for the validation of bioanalytical methods with 1) an accuracy mean value within +15% of the nominal value; 2) the within-assay precision less than 7.1%, and 3) the inter-day variability under 12.1%. With the A2HRP method, it is then possible to quantify from 0.04×10^{12} to 2.98×10^{12} functional Ab binding sites immobilized on the solid surfaces. A2HRP method was validated according to FDA and EMA guidance, allowing the creation of a gold standard method to evaluate Ab surfaces for their resistance under laboratory constraints. Stability testing was described through forced degradation studies after exposure of Ab-surfaces to storage, pH and aqueous-organic solvent mixture stresses.

1. Introduction

Antibody (Ab)-based materials are affinity ligand-based solid-state devices using the specificity of the molecular recognition of antigens (Ag) by antibodies to form a stable complex. Ag-Ab interactions permit to selectively isolate or analyze target components within a crude preparation for clinical and biomedical applications but also in environmental contaminants analysis. In these formats as for the common enzyme-linked immunosorbent assay (ELISA) one, the key step is the coating of the solid surface with Ag or Ab to allow direct, indirect *vs*. capture or sandwich detection. The first requirement is to have a final uniform coated surface to prevent the unspecific adsorption of other components of the samples, or of the primary/secondary Ab used during the detection step. Otherwise, the assay performances may be greatly reduced due to loss in specificity. The important feature that the immobilization step should accomplish is to keep the functionality of the binding partners without affecting their three-dimensional structure since both uniformity of surface coating and Ag-Ab interactions accessibility will give the final sensitivity of the assay. So, reliability refers to the sensitivity and specificity of the assay.

Immunospecific assays for the direct detection of horseradish peroxidase (HRP) based on similar principles to ELISA but without the requirement of an enzyme-conjugated Ab have been proposed [1–5]. In recent times, HRP/anti-HRP Ab was used as model for recognition system to evaluate chip functionality [2]. Based on

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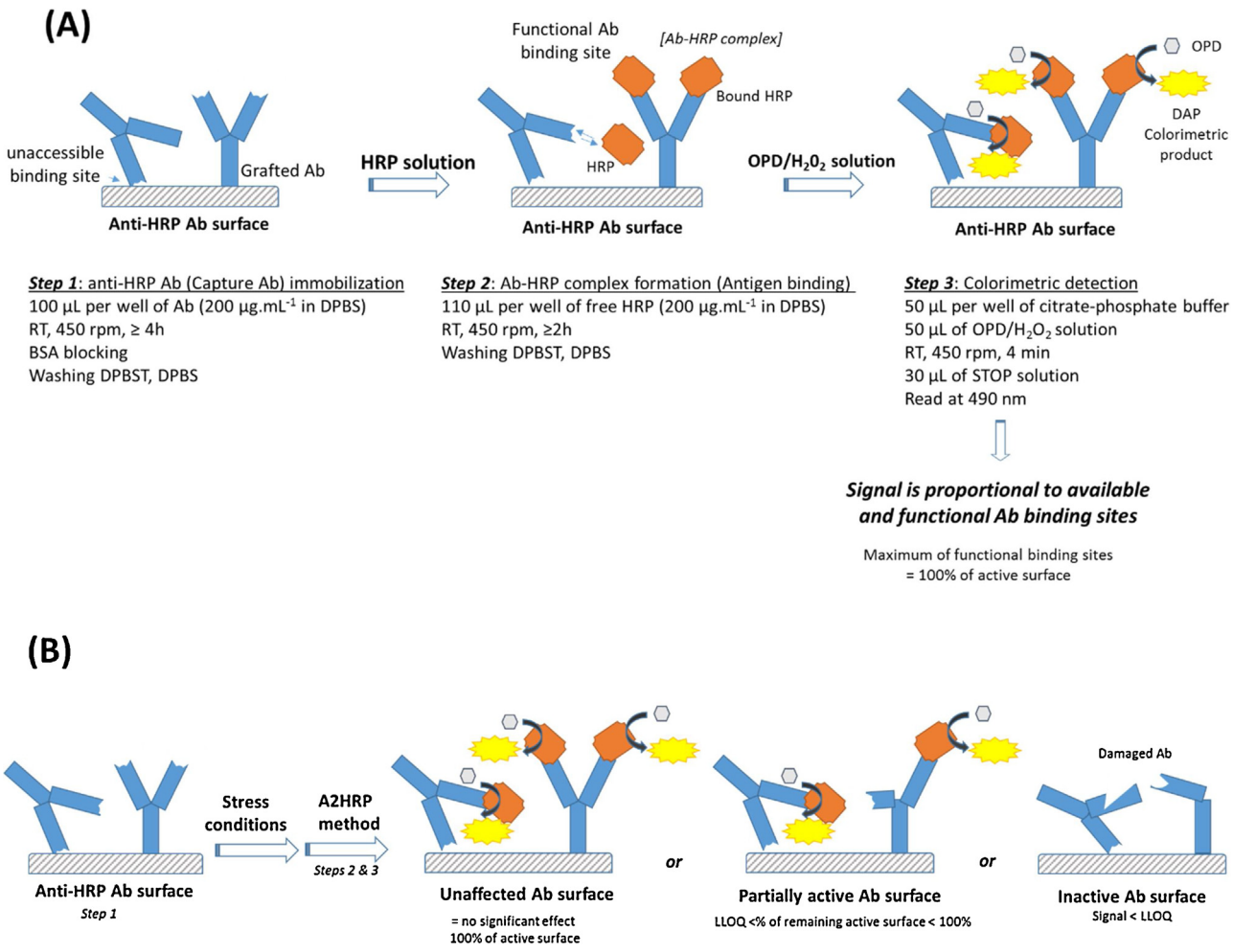

Fig. 1. A2HRP method. (A) The A2HRP principles and its optimized protocol that was validated in the present paper and its potential use (B) in determining if Ab surface was (partially or totally) affected or not after undergoing laboratory stress conditions.

this principle, our group proposed a method called Antibody Anti-HorseRadish Peroxidase method, A2HRP method (Fig. 1). While the concept of the proposed methodology is simple, the method was technically challenging to develop because both HRP and Ab activities necessitated to be preserved during the assay. The choice of the immobilization strategy (covalent grafting) and the optimization of buffers/reagents used during recognition and detection steps have already been performed in our group [6]. In these studies, we reported the influence of the Ab immobilization process on the Ab surfaces resistance to short-term events (freeze-drying, temperature shifts) [6,7]. To ensure A2HRP method consistency over the time frame required for multi-months stability in the context of clinical studies, or in other fields such as space exploration, we intended to evaluate the performances of the A2HRP method by changing (bio)reagent lots, over a predetermined amount of time. Indeed, possible variability can be observed, in the resulting density of chemical groups of pre-activated coated plates, in anti-HRP antibodies production lot and in HRP preparations, the data might be variable over time.

The entire validation of the A2HRP method was performed according to the EMA (European Medicines Agency), Food Drug Administration (FDA), and International Council for Harmonisation (ICH) guidelines for (bio)analytical method validation [8–10]. Working range, selectivity, accuracy, precision, and the quantification limit of the A2HRP method (Lower Limit of Quantification, LLOQ) are reported hereafter.

2. Materials and methods

2.1. Chemical, reagents and materials

Mouse monoclonal anti-horseradish peroxidase antibodies (anti-HRP Ab) were obtained from MyBioSource (clone number B215M, lot 2F15911 *used during validation process*, lot 2F17811, USA). Anti-Mouse IgG (Fc specific)-Alkaline Phosphatase (AP) conjugated antibody produced in goat (lot 084M4781V), BSA (bovine serum albumin for biochemistry, fraction V, 96–100% protein, lot SLB38588V *used during validation process*, lot SLBN2232V, SLBN3564V), Horseradish peroxidase (HRP, EC 1.11.1.7, lot SLBF8268V *used during validation process*, lot SLBC2881V, lot SLBQ1119V) with a purity index (RZ) of 1.9), Tween® 20 (impurities ≤3% in water, lot A017826501), *o*-phenylenediamine dihydrochloride (OPD-2HCl, lot 091M5309V *used during validation process*, lot SLBC3317V, lot SLBM6498V), *p*-nitrophenylphosphate (pNPP, in tablets format SigmaFast™ lot SLBK4199V), hydrogen peroxide (H_2O_2, 30%, (v/v) solution, lot MKBQ0465V *used during validation process*, lot MKBQ6981V), and Dulbecco's Phosphate Buffered Saline (DPBS, 10X solution, pH 7.4, used in a final concentration of 1X in water solvent, lot RNBD6083 *used during validation process,* lot RNBD2525, lot RNBF6338), sulfuric acid (H_2SO_4, ≥97,5%, lot SZBB1590V), citric acid ≥99,5%, hydrochloric acid (HCl, 37%), glycine hydrochloride (glycine-HCl, 98%), sodium bicarbonate ($NaHCO_3$) ≥99,5%, sodium carbonate (Na_2CO_3) ≥99,5% were purchased from Sigma Aldrich (Saint-Quentin Fallavier, France).