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Research paper

Affinity capture elution bridging assay: A novel immunoassay format for detection of anti-therapeutic protein antibodies

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ABSTRACT

Background: Increased emphasis on the development of biologics has placed a significant focus on anti-drug antibody (ADA) detection. To address this need, several immunoassay formats have been described for use in characterizing potential immune responses. Two commonly utilized methods include the affinity capture elution (ACE) and bridging formats. While these approaches have been effective in supporting many clinical initiatives, both possess potential disadvantages. Here, we compare these standard methods to a novel format that addresses these noted drawbacks.

Results: A novel assay format has been designed to incorporate the benefits of the ACE and bridging methods while overcoming the disadvantages incurred with each approach. The described ACE–Bridge format exhibits excellent sensitivity and precision while providing superior drug tolerance when compared to bridging formats. Further, this assay format is not susceptible to the endogenous target interference that can be an issue in the ACE format.

Conclusions: The ACE–Bridge format provides an often superior option as a screening method to monitor patient ADA responses. This method is unique in its ability to measure ADA in the presence of high circulating endogenous target concentrations (>100 ng/mL) while demonstrating very high drug tolerance.

1. Introduction

All large molecule drug products currently in development carry the risk of initiating an immune response in the patient (Beck et al., 2010; Nelson et al., 2010). Multiple factors can influence the immunogenicity of therapeutic proteins including: size, purity, the degree of sequence homology, manufacturing processes, formulation, dose level and dosing frequency (Van Walle et al., 2007; Koren et al., 2008). Further, the patient health status and condition of the immune system, as well as the possibility of co-administration of other drugs such as immunosuppressive therapy may also impact potential immunogenicity outcomes. An immune response to a therapeutic protein may result in the elicitation of anti-drug antibodies (ADAs). This response can give rise to outcomes ranging from no adverse effect to deleterious outcomes due to the decreased efficacy or altered PK of the drug product. Neutralizing antibodies (NAb) engage therapeutic proteins and negate their ability to bind the target antigen; thus, NAb can potentially alter the efficacy of the therapeutic. Even more concerning, the potential for neutralizing an endogenous protein is a possibility in the event that cross reactive ADAs are made to a therapeutic protein. Therefore, it is necessary to develop and validate methods that detect and confirm the presence of ADA that can bind and may neutralize the activity of the therapeutic proteins (FDA, 2009). The performance characteristics of several different screening immunogenicity formats have been previously compared (Liang et al., 2007; Lofgren et al., 2007; Butterfield et al., 2010; Menendez, 2012). Here, we introduce a novel and improved approach to ADA screening that alleviates shortcomings that have plagued other formats.

Testing for ADA is typically carried out in a tiered approach, employing a screening immunoassay as a primary test for the detection of binding ADA (Mire-Sluis et al., 2004; FDA, 2009). In this approach, samples are screened (tier 1) for ADA binding, then putative positive samples (samples running above the determined cut point) are run in a confirmatory assay (tier 2). Confirmed positive samples are then titered (tier 3) for relative quantitation of the ADA response. Finally in tier 4, positive samples are assessed for the presence of NAb.

Humanized monoclonal antibodies exhibit half-lives of 10–20 days and can be administered at high doses, potentially resulting in circulating therapeutic concentrations greater than 500 μg/mL (Wang et al., 2008). Bridging format assays are often used in the evaluation of ADA against therapeutic monoclonal antibodies (TAbs) (Moxness et al., 2005; MesoScaleDiscovery, 2011). The bridging assay format requires bivalent ADA binding to labeled therapeutics, and as a result,

Abbreviations: MSD, MesoScale Discovery; ECL, electrochemiluminescence; ACE, affinity capture elution; ADA, anti-drug antibody; TAb, therapeutic antibody; MRD, minimum require dilution; TMB, 3,3′,5,5′ tetramethylbenzidine; HRP, horseradish peroxidase; NHS, normal human serum; TBS, Tris-buffered saline; SEM, standard error of the mean.

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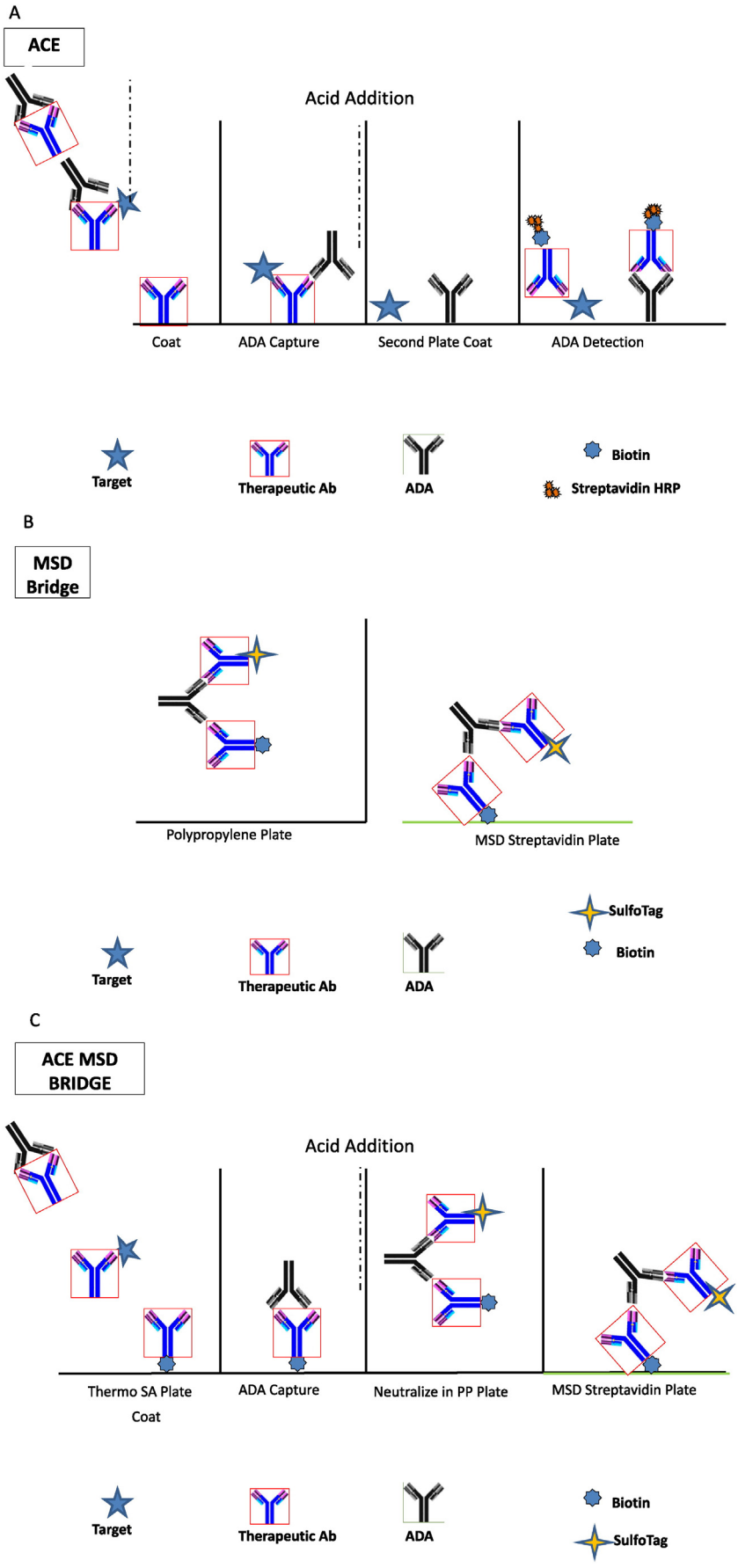

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