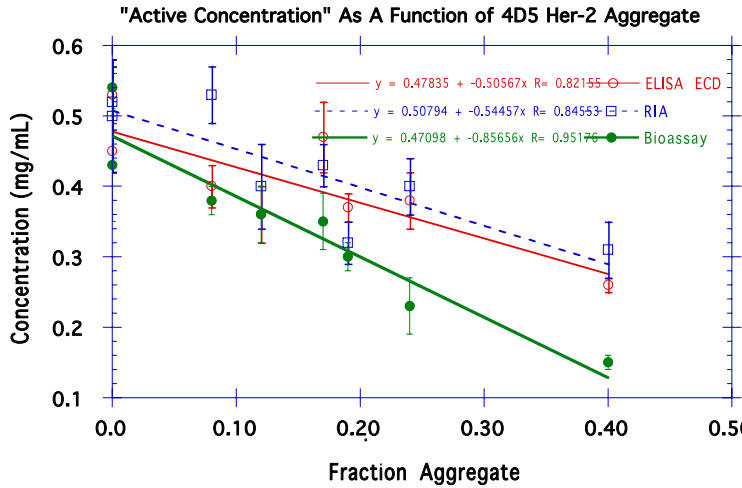

Figure 2.6 Active concentration of 4D5 HER2 murine mAb determined by ELISA binding to the extracellular domain (ECD) of p185HER2, RIA binding to ECD, and the bioassay as a function of aggregate content.

allergen. The IgE binds via its Fc region to high-affinity receptors on basophils and mast cells. These cells are then primed and upon a second exposure to the allergen results in cross-linking of the IgE bound to the high-affinity Fc receptors. The cross-linking then signals the mast cell or basophil to release histamine and leukotrienes that result in asthmatic symptoms. An anti-IgE mAb that binds to essentially the same site on the IgE as the high-affinity receptor prevents the initial priming of the cell. An in vitro assay that mimics this mechanism of action was done using ELISA. In this assay a 96-well plate is coated with an FcεRIα–IgG chimeric receptor. The chimeric receptor essentially is an IgG Fc fused with two soluble forms of the FcεRIα extracellular domains. A mixture of IgE and biotin-labeled IgE is added with different amounts of the anti-IgE mAb (Figure 2.7(a)). The binding to the FcεRIα–IgG chimeric was then assessed using avidin conjugated to horseradish peroxidase (HRP) followed by detection using a substrate that, when oxidized by HRP using hydrogen peroxide as the oxidizing agent, yields a characteristic change that is detectable by spectrophotometric methods. In another assay the amount of biotin-labeled IgE bound to the FcεRIα–IgG chimeric was determined. A bioassay that uses allergen-induced histamine release from mast cells transfected with the α-subunits of FcεRI was also developed as an ex vivo potency assay (Lowe, Jardieu, VanGorp, & Fei, 1995). These three assays were used to analyze percent specific activity (the effective concentration from the assay divided by the concentration determined spectrophotometrically) of anti-IgE mAb stored at −70 °C, 2–8 °C, 25 °C, and 40 °C over 36 weeks of storage. The correlation of the receptor binding inhibition assay with the bioassay was much better than that of the IgE plate binding assay (Figure 2.7(b)). While this example again shows the danger of using a straight binding assay it also shows that formatting the assay in a competitive binding mode can result in development of an in vitro surrogate assay for the bioassay. This approach worked since the mechanism of action biologically is replicated with the in vitro receptor binding inhibition assay.