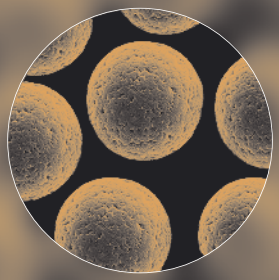
Dynabeads® Tosylactivated Optimized for Immunoassay IVD

Improved Dynamic Range in a D-Dimer Model System



Introduction

The use of magnetic beads as the solid phase in automated immunoassay instruments is a well-established procedure which is being adopted ever more frequently due to their superior sensitivity. The introduction of different magnetic solid phases into existing immunoassays is relatively simple as the requirements for optimization studies are comparatively minimal.

Here the performance of two different Dynabeads® are compared. Both Dynabeads® MyOne™ Tosylactivated and Dynabeads® M-280 Tosylactivated coated with capture-antibody using an optimized procedure are compared to Dynabeads® M-280 Tosylactivated coated with the standard procedure. All beads are used as a solid phase in an immunoassay for D-Dimer.

The immunoassay for D-Dimer is used as a model system. D-Dimer is a 180 kD final product of cross-linked fibrin degradation and elevated plasma levels are indicative of a prolonged fibrinolytic process. Using Dynabeads® Tosylactivated in a D-Dimer Immunoassay model system exemplifies the performance which can be expected for antigens having similar characteristics.

Materials and Methods

For preparation of the solid-phase capture reagents, Dynabeads® MyOne™ Tosylactivated and Dynabeads® M-280 Tosylactivated were coated with an antibody against D-Dimer, *clone C1*, using an optimized coating procedure. For comparison, Dynabeads® M-280 Tosylactivated were coated with the same antibody, but following the standard coating procedure. In the subsequent text, these coated beads will be referred to as *"standard beads"*.

Standard beads and the Dynabeads® M-280 Tosylactivated with the optimized coating procedure were used at a concentration of 1.25 mg/ml. Various concentrations of Dynabeads® MyOne™ were used in order to ascertain the concentration that gave a similar or improved performance when compared to the *standard beads*. The amount of Dynabeads® MyOne™ used was 30, 40 and 50 % weight of that used with the *standard beads*.

For detection, an antibody, *clone D1*, was conjugated with the chemiluminescent tag, isoluminol. An automated random access bench top analyzer was used. The concentration of D-Dimer is positively correlated with the signal in "relative light units" (RLU).

The assay is a sequential sandwich immunoassay in which the first step is incubation of sample with the solid phase for capture, followed by a wash step to remove unbound sample and impurities. The detection antibody is then added and incubated, before a final wash step and subsequent detection of signal. Standards and samples were citrated plasma. Washing and incubation buffer was PBS with 0.1% BSA.

A standard curve measurement was performed using standards from 0 to 18,000 ng D-Dimer/ml. Signal to noise ratio was determined by the RLU of first standard (25 ng/ml) divided by background RLU (zero standard). Dynamic range is determined as the span of curve, or the RLU of the highest standard (18,000 ng) divided by background RLU.

Samples of citrated plasma with three different D-Dimer concentrations were analyzed to compare recoveries. In order to investigate the robustness of the assay, a sample with a high concentration of D-Dimer was diluted both 5 fold and 20 fold and all dilutions analyzed. In a robust assay, parallelism should be in the range of 80-120 % of the undiluted sample.

Results

The standard curves for the various Dynabeads®, and at the different bead concentrations for Dynabeads® MyOne™ , in the D-Dimer immunoassay are described in fig. 1. Comparison of the curves achieved with the *standard beads* and Dynabeads® M-280 with the optimized coating procedure, demonstrates that an optimized coating procedure improved the assay performance. Also, titration of the Dynabeads® MyOne™ demonstrates that the same signal can be achieved with lower bead concentrations.

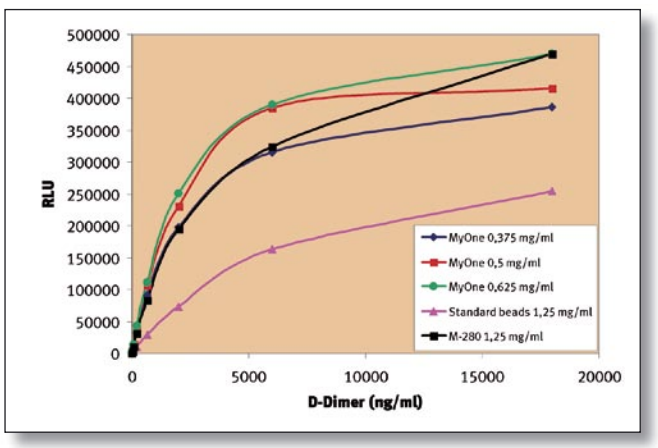


Fig. 1: Standard curve measurements for a D-Dimer immunoassay as measured on an automated random access bench top analyzer. Bead concentrations for Dynabeads® MyOne™ of 0,375 mg/ml, 0,5 mg/ml and 0,625 mg/ml corresponds to 30 %, 40 % and 50 % weight of Dynabeads® M-280.

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The results for signal to noise ratios and span of curve are given in table 1 and demonstrate that the optimized coating procedure had also improved these assay variables. Results of the analyses of the spiked samples are also shown in table 1, and very similar results were obtained for all beads used for the solid phase capture.

	Dynabeads® MyOne™	Dynabeads® M-280	*Standard Beads*
Bead concentration	0.5 mg/ml	1.25 mg/ml	1.25 mg/ml
Signal to noise ratio	12,2	11,7	4,1
Span of curve	1119	1579	768
Sample low level	156 ng/ml	118 ng/ml	155 ng/ml
Sample medium level	544 ng/ml	482 ng/ml	524 ng/ml
Sample high level	1937 ng/ml	2177 ng/ml	2032 ng/ml

Table 1: Comparisons of signal to noise ratios, spans of curves, and recovery results when using standard beads or Dynabeads® M-280 and Dynabeads® MyOne™ with optimized coating.

The robustness of the assay, as measured by parallelism of results after dilution of a sample, is shown in table 2. Similar results were obtained for all beads, demonstrating that the optimized coating protocol yields very robust assays for both Dynabeads® M-280 and Dynabeads® MyOne™.

	Dynabeads® MyOne™	Dynabeads® M-280	*Standard Beads*
High level sample	4258 ng/ml	3881 ng/ml	3306 ng/ml
Dilution 1:5	924 ng/ml	860 ng/ml	871 ng/ml
Parallelism	109 %	111 %	132 %
Dilution 1:20	234 ng/ml	209 ng/ml	224 ng/ml
Parallelism	110 %	108 %	136 %

Table 2: Robustness of the D-Dimer immunoassay using the 3 bead types, measured as parallelism of results following analysis of samples containing high concentrations of D-Dimer, and two dilutions of that sample.

Discussion

The shape of the standard curves with the different beads used in the solid phase capture demonstrates that when an optimized coating procedure is used for the capture antibody, the results are markedly improved.

Additionally, the optimized coating procedure results in the signal to noise ratio increasing markedly, from approximately 4 with the *standard beads*, to approximately 12 for both Dynabeads® M-280 and Dynabeads® MyOne™. The span of curve is similarly improved.

When smaller beads are used in an assay, number of beads per volume weight is higher. This factor of increased bead number, together with comparative surface area of the different bead sizes, correlates well with the requirement for 60% less weight of beads when1 µm beads (Dynabeads® MyOne™) are used rather than 2.8 µm beads (Dynabeads® M-280).

When considering immunoassay conditions, the number of beads per volume weight used is important, as this can influence the reaction kinetics. Previous research has demonstrated that increasing bead number results in faster reaction kinetics until a plateau is reached, and that to some extent, this is independent of bead size. Thus, when smaller magnetic beads are used, it is possible to achieve high sensitivity with lower incubation times.

Features and Benefits of Dynabeads® in Immunoassays

Dynal Biotech's proprietary technology is the production of monodisperse superparamagnetic Dynabeads®. Their quality is renowned in the in vitro diagnostic immunoassay market and they fulfill critical and important criteria for use as the solid phase in immunoassays:

- High capacity.
- High signal to noise ratio.
- Easy development of assays.
- Efficient and reproducible immobilization of antibodies (or other ligands).
- Easy handling in manufacturing.
- Reproducible behavior in automation.

Conclusions

Using an immunoassay for D-Dimer as a model system, we have demonstrated that tosylactivated Dynabeads® of two different sizes, and with optimized capture antibody coating, can be readily introduced into an existing immunoassay, giving improved signal to noise ratios as well as increased dynamic range. Both sizes of tosylactivated Dynabeads® are suitable for immunoassays; choice of bead size will also be dependent on instrument characteristics such as geometry of reaction wells, magnetic separation properties, and mixing possibilities.

The work has been carried out in collaboration with Future Diagnostics, bv.

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