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Accuracy and Dynamic Range of Thermo Scientific Nunc MaxiSorp Plates: Competitive Comparison

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Key Words: Nunc MaxiSorp Surface, ELISA, Dynamic Range, Plate Accuracy.

Goal

The goal of this study was to compare the dynamic range and plate accuracy of Nunc™ MaxiSorp™ Immuno Assay Plates with those of competitors.

Abstract

Assay accuracy, and the range of concentrations over which the assay is accurate, is an important factor in deciding on a plate brand to use in immuno assays. Here we compare the accuracy and the dynamic range of 11 plates of several brands for use in ELISA using a commercially relevant assay. Nunc MaxiSorp plates demonstrated the widest dynamic range (4-2000 pg/mL of the protein tested) of all plates tested. Nunc MaxiSorp plates also showed better reproducibility, with 100% of plates exceeding an R^2 value of 0.99, and therefore the most accurate results of all plate brands tested.

Abbreviations

BSA: Bovine serum albumin
ELISA: Enzyme-linked immunosorbant assay
**HRP:** Horseradish peroxidase
O.D.: Optical density
PBS: Phosphate buffered saline
TNF-alpha: Human tumor necrosis factor-alpha

Introduction

In quantitative tests such as an enzyme-linked immunosorbant assay (ELISA), it is important to know not only the minimum concentration of reagent the test will detect, but also the test accuracy and range of concentrations over which the test is accurate.
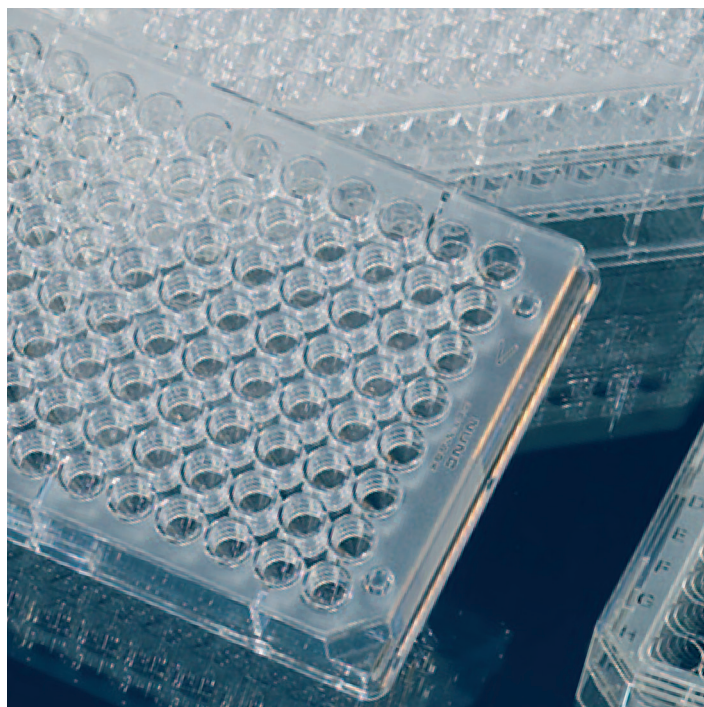
Since many ELISAs are performed using disposable multi-well plates, the properties of the polystyrene plate could alter the binding properties of reagents in the solid phase portion of the assay. This in turn could affect the accuracy of the plates at different concentrations of the reagent being tested. The range of concentrations over which the plate is accurate (dynamic range), and the reproducibility of the dose-response curve, are important determinants for the end user when deciding the brand of plates to utilize in testing. Here we examine the accuracy and dynamic range of Nunc MaxiSorp plates compared to competitors' products when used in ELISA tests.

Thermo Fisher
S C I E N T I F I C

Materials	
Duo ELISA kit, human TNF-alpha /TNFSF1A:	R&D Systems, Cat #DY210, lot #1255911EAJ and #1276651EC
Recombinant human TNF-alpha:	R&D Systems, Cat #210-TA-050, lot #AA3511011
Bovine serum albumin (BSA):	Fisher, Cat #BP-1600-100, immunology grade
Sodium chloride:	Fisher #S271-1, fw 58.44, lot #084362
Potassium chloride:	Sigma #P-9541, fw 74.55, lot #51K0146
Sodium phosphate dibasic:	Sigma, Cat #S0876-500g, fw 141.96, lot #040M0187
Potassium phosphate monobasic:	Fisher, Cat #P285-500, fw 136.09, lot # 073345
Citric acid monohydrate:	Fisher, Cat #A104-500, fw 210.14, lot #041657
Hydrogen peroxide, 30% solution:	Mallinckrodt, Cat #5240-02, fw 34.01, lot #H28300
o-phenylenediamine dihydrochloride (OPD):	Sigma, Cat #P8412-100TAB, 30 mg/tablet, lot #070M8215
2N sulfuric acid:	RICCA, Cat #8310-32, lot #2108222
Spectrophotometer:	Thermo Scientific™ Varioskan™ Flash system
Titertek:	MAP-C2, Cat #52045

Methods

Reagents from a commercially relevant ELISA kit were prepared as per kit instructions as follows: 0.1 mL capture antibody solution (diluted to 4 µg/mL) was added to each well of the plate, and plates were sealed and left overnight at room temperature. Plates were washed three times, then blocked for one hour with 0.3 mL reagent diluent (10 g/L BSA in PBS) in each well, and washed again. Human TNF-alpha was prepared in serial 1:2 dilutions with the reagent diluent from 2000 pg/mL to 2 pg/mL.

Sensitivity tests were conducted to determine the minimum detectable concentration for each plate brand. TNF-alpha concentrations were distributed on the plates as in Table 2. 0.1 mL of diluted TNF-alpha solution was added to each well and incubated for two hours at room temperature. After wash, 0.1 mL of detection antibody (diluted to 250 ng/mL) was added to each well and incubated for two hours at room temperature. Following wash, 0.1 mL diluted streptavidin-HRP (1:200 dilution) was added to each well and incubated for twenty minutes at room temperature. Following a final wash, 0.1 mL substrate solution was added, incubated 20 minutes, and then 0.05 mL stop solution was added. Incubation time of substrate solution was precisely controlled to maintain consistency across plates. Optical density (O.D.) of plates was read using a spectrophotometer at 492 nm wavelength, 5 nm gap, 100 ms settling delay, and 300 ms measurement time. Detection threshold was calculated at two standard deviations above the mean of optical densities for blank wells (without TNF-alpha) in each plate. Plate sensitivity was recorded as the minimum concentration of antigen that produced an O.D. that exceeded the detection threshold.

Dynamic range tests were conducted to determine the range of concentrations that produced accurate results for each plate. Procedures were followed as above, except using the test plate plan shown in Table 3. Specific optical density (specific O.D. = measured O.D. - blank O.D.) for wells in each numbered column were averaged for each plate. The resulting means were plotted as a dose-response curve on a log-log axis and an R^2 calculation was performed in Excel (Microsoft Corporation, RSQ function) to determine linearity.

$$R^2 = \left(\frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2 \ \sum(y-\bar{y})^2}} \right)^2$$

where x = protein concentration and y = O.D.

	Manufacturer / Brand	Plate Name
1	Thermo Scientific	Nunc MaxiSorp, F96 (Product #442404)
2	Thermo Scientific	Nunc™ MediSorp™, F96 (Product #467320)
3	Competitor A	High binding, low volume well strips, 96 wells
4	Competitor A	High binding well strips, 96 wells
5	Competitor B	High binding 96 well plate
6	Competitor C	Extra-high binding 96 well plate
7	Competitor C	High binding 96 well plate
8	Competitor D	High binding 96 well plate
9	Competitor E	Enhanced binding 96 well plate
10	Competitor F	High binding 96 well plate
11	Competitor G	High binding 96 well plate

Table 1. Immuno-plate sample manufacturer and type

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	0	0	0	0	0	0	0	0	0	0	0	0
C	2	2	2	2	2	2	2	2	2	2	2	2
D	4	4	4	4	4	4	4	4	4	4	4	4
E	8	8	8	8	8	8	8	8	8	8	8	8
F	16	16	16	16	16	16	16	16	16	16	16	16
G	31	31	31	31	31	31	31	31	31	31	31	31
H	63	63	63	63	63	63	63	63	63	63	63	63

Table 2. Test plate plan indicating TNF-alpha concentration distribution (pg/mL) for sensitivity tests.

R^2 in this case represents the correlation between the actual protein concentration in solution and the resulting O.D. measured, or plate accuracy; a value of 1 corresponds to perfect correlation. The minimum concentration found in sensitivity testing for each plate was recorded as the lower limit of the dynamic range. The upper limit of the dynamic range was defined as the maximum concentration for which the calculated R^2 value remained above 0.98. Three plates of each brand were tested. Values were converted to logarithmic values *(logarithmic = ln[upper limit])* and averaged within each plate manufacturer/type.

These means were then converted back to arithmetic values *(average upper limit = $e^{\text{logarithmic mean}}$)* to produce an average upper limit of the dynamic range for each plate manufacturer. The results of nine additional plates of each brand (eight for one type of plate from Competitor A) with varying antigen concentrations were plotted as in dynamic range testing, but only covering the range of concentrations recommended by the ELISA kit manufacturer (16-1000 pg/mL). These results were combined with the dynamic range test results, and R^2 values were calculated for all plates to determine plate accuracy.

Results and discussion

The average sensitivity was calculated for all plates to determine the lower limit of dynamic range for each brand. Nunc MaxiSorp plates averaged near the lower portion of the range of sensitivities for all competing plates, at 4 pg/mL. Table 4 represents a sample data set containing all of the specific O.D. (measured O.D. minus blank O.D.) values for one Nunc MaxiSorp plate. The mean values at each concentration were plotted in the graph seen in Figure 1.

The R^2 value of the linear regression of mean values was calculated at 0.9876 over the full range of values (4-2000 pg/mL). The upper limit of the dynamic range for this plate was therefore determined to be 2000 pg/mL. The average of all three plates for Nunc MaxiSorp plates was 2000 pg/mL, the highest of all plates tested. The upper limit of the dynamic range can be seen for all manufacturers in the third column of Table 5.
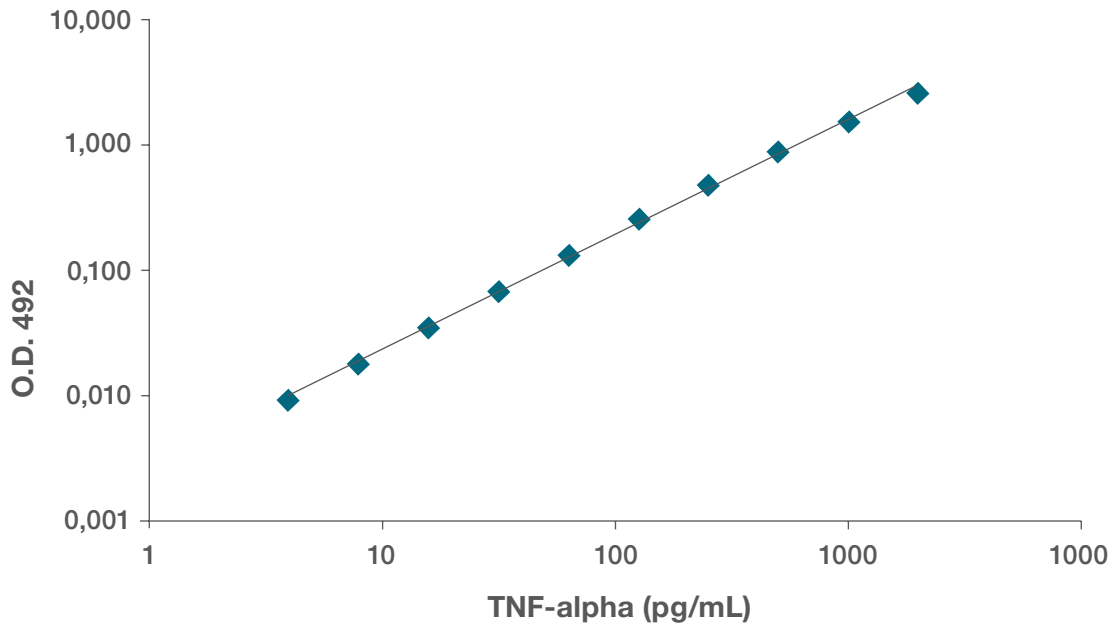

Figure 1. Sample dose-response curve plot of data from Table 4 showing means of each concentration plotted as data points and the regression line. The calculated R^2 value for the regression line is 0.9876 over the range of all data points plotted.

	1	2	3	4	5	6	7	8	9	10	11	12
A		0	4	8	16	31	63	125	250	500	1000	2000
B		0	4	8	16	31	63	125	250	500	1000	2000
C		0	4	8	16	31	63	125	250	500	1000	2000
D		0	4	8	16	31	63	125	250	500	1000	2000
E		0	4	8	16	31	63	125	250	500	1000	2000
F		0	4	8	16	31	63	125	250	500	1000	2000
G		0	4	8	16	31	63	125	250	500	1000	2000
H		0	4	8	16	31	63	125	250	500	1000	2000

Table 3. Dynamic range test plate plan indicating TNF-alpha concentration distribution (pg/mL).

	1	2	3	4	5	6	7	8	9	10	11	12
A		0.005	0.012	0.022	0.045	0.083	0.155	0.286	0.532	0.57	1.626	2.673
B		-0.003	0.009	0.016	0.031	0.066	0.134	0.257	0.490	0.909	1.594	2.692
C		-0.006	0.007	0.015	0.034	0.064	0.124	0.247	0.477	0.890	1.511	2.548
D		-0.004	0.005	0.016	0.029	0.057	0.118	0.238	0.455	0.854	1.465	2.561
E		-0.002	0.008	0.014	0.030	0.063	0.122	0.244	0.470	0.872	1.493	2.571
F		0.000	0.006	0.015	0.029	0.065	0.129	0.250	0.457	0.866	1.472	2.498
G		0.002	0.007	0.017	0.035	0.072	0.131	0.256	0.485	0.890	1.547	2.651
H		0.007	0.019	0.026	0.041	0.079	0.143	0.279	0.518	0.979	1.707	2.787
TNF-α (pg/mL)		0	4	8	16	31	63	125	250	500	1000	2000
Mean		0.000	0.009	0.018	0.034	0.069	0.132	0.257	0.486	0.902	1.552	2.623
SD		0.004	0.004	0.004	0.006	0.009	0.012	0.017	0.027	0.044	0.085	0.095

Table 4. Sample data from one Nunc MaxiSorp plate, corrected to the average of blank wells (measured O.D. – mean blank O.D.). Mean blank O.D. = 0.112.

Manufacturer / Brand	Plate	Mean Accurate Maximum Concentration	Percent of Plates with R^2 value:		
			> 0.99	0.98 to 0.99	< 0.98
Thermo Scientific	Nunc MaxiSorp, F96	2000	100.0	0.0	0.0
Thermo Scientific	Nunc MediSorp, F96	1260	83.3	8.3	8.3
Competitor A	High binding, low volume well strips, 96 wells	1587	91.7	8.3	0
Competitor A	High binding well strips, 96 wells	794	75	16.7	8.3
Competitor B	High binding 96 well plate	1000	41.7	50.0	8.3
Competitor C	Extra-high binding 96 well plate	1260	81.8	18.2	0.0
Competitor C	High binding 96 well plate	1587	83.3	8.3	8.3
Competitor D	High binding 96 well plate	1000	41.7	33.3	25.0
Competitor E	Enhanced binding 96 well plate	1260	75.0	16.7	8.3
Competitor F	High binding 96 well plate	1000	58.3	33.3	8.3
Competitor G	High binding 96 well plate	630	33.3	33.3	33.3

Table 5. Summary of results for plates from all manufacturers. Nunc MaxiSorp plates had the highest average maximum concentration with R^2 > 0.98, at 2000 pg/mL. Maximum concentration values are calculated as in Table 4 and Figure 1. Percentage of plates with various R^2 values in rightmost columns were calculated based on the dose-response curves from 16 to 1000 pg/mL.

R^2 values from dynamic range tests (limited to 16-1000 pg/mL) were combined with results from additional plates of each brand. The percentage of plates under three conditions was recorded: with an R^2 value greater than 0.99, R^2 between 0.98 and 0.99, and R^2 less than 0.98. Results for all plates are found in Table 5. A high percentage of plates with dose-response curve R^2 values greater than 0.99 indicates a high degree of accuracy for that brand of plate. 100% of R^2 values for Nunc MaxiSorp plates were greater than 0.99, indicating a very high degree of accuracy for these plates.

Conclusion

The Nunc MaxiSorp plates had the highest average upper limit of dynamic range, and consequently the largest dynamic range (4-2000 pg/mL) of all the plates tested. These plates also best reproduced the linearity of the dose-response curve over the range of antigen concentrations specified by the kit manufacturer, with 100% of plates exceeding an R^2 value of 0.99. This linearity indicates that the plates produce highly accurate results reflecting the known concentrations under the test conditions. The test accuracy indicated by this reproducibility is important in choosing a plate for use in ELISA testing, as plates that more accurately reflect antigen concentration will produce more accurate results in everyday use. Nunc MaxiSorp plates are therefore the ideal plate for use in assays that require very accurate measurements over a wide range of antigen concentrations.

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