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Improved Reproducibility of ELISA Using a Static- Discharge Pad

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While performing enzyme-linked immunosorbent assays (ELISAs) we have occasionally noted "geographical" effects on the ELISA plates, in which results from one region of the plate were considerably lower than those obtained on a different part of the plate. This effect would occur spontaneously, persist for a variable period of time and then disappear. Among the explanations we considered and excluded were variations in plate or reagent temperature, pipeting errors, unequal plate washing and residual reagent remaining in the

wells. We became suspicious that static electricity might be affecting our results after observing the plastic wrap covering the plates was statically charged. Therefore, we set out to determine if an ELISA plate, discharged of static electricity at each preparation step, yielded better results as compared with a plate similarly prepared on a bench top.

Two ELISA plates (Immunolon® 2, U-Bottom; Dynatech Laboratories, Chantilly, VA, USA) were similarly prepared, either exclusively on a static-discharge pad (discharged plate) or on a laboratory bench top (bench-top plate). The static-discharge pad (Computer Gate International, Santa Clara, CA, USA) conducts static electricity away from the ELISA plate by means of a connection to the ground plug of an electrical outlet. Further, the user is grounded by a wristband connected to the discharge pad.

The inner 60 wells of each plate were coated with the antigen, maltose-binding protein (MBP), a product of the pMAL-c2 plasmid (New England Biolabs, Beverly, MA, USA). Antigen was added at 0.2 µg/mL in a volume of 0.1 mL. Both plates were wrapped in Saran® Wrap plastic film and incubated overnight at 4°C. (For this and all subsequent steps, the plastic wrap covering the discharged plate was discharged on the static-discharge pad). The wells were then blocked overnight at 4°C using phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA) and 0.1% sodium azide in a total volume of 0.2 mL. The ELISA was performed by adding rabbit anti-MBP antiserum, 1:10 000, 0.1 mL to all wells (New England Biolabs). After an overnight incubation at 4°C, the plates were extensively washed using PBS supplemented with 0.1% Tween® 20 (PBS/Tween 20). The plates were then incubated with alkaline phosphatase (AP)-conjugated goat anti-rabbit antibody, 1:1000, 0.1 mL in each well (Zymed Laboratories, South San Francisco, CA, USA) for 4 h at room temperature. The plates were washed extensively using PBS/Tween 20 and developed using *p*-nitrophenyl phosphate (Sigma Chemical, St. Louis, MO, USA) dissolved in 9.8% diethanolamine, 0.5 mM MgCl₂. Ninety minutes later, the optical density (OD)

Benchmarks

A)										
	2	3	4	5	6	7	8	9	10	11
B	1.9	1.9	1.9	2.0	1.9	2.0	2.1	2.0	2.0	2.0
C	1.9	1.8	1.8	1.8	2.1	2.0	2.1	1.9	2.0	1.9
D	1.9	1.7	1.8	1.9	1.9	1.9	1.8	2.0	1.9	2.0
E	1.8	1.8	1.9	1.9	1.9	1.9	2.0	1.9	1.8	2.0
F	1.8	1.8	1.8	2.0	1.9	2.1	2.0	2.3	2.0	2.1
G	1.8	1.9	1.8	2.0	2.1	2.1	2.0	2.2	2.1	2.1

B)										
	2	3	4	5	6	7	8	9	10	11
B	2.1	2.4	2.4	2.4	2.4	2.2	1.9	1.6	1.0	0.5
C	2.0	2.4	2.4	2.4	2.4	2.3	1.9	1.1	1.0	0.6
D	2.0	2.2	2.3	2.3	2.2	2.1	1.4	1.1	0.6	0.4
E	2.1	2.2	2.3	2.3	2.3	2.1	1.6	1.4	1.1	0.5
F	2.0	2.2	2.2	2.3	2.2	2.1	1.7	1.4	0.9	0.5
G	2.1	2.4	2.2	2.4	2.1	2.1	1.4	1.1	0.5	0.4

Figure 1. OD values of plates prepared either on a bench-top or static-discharge pad. The geographical location of individual wells is indicated. The plates were prepared either on (A) a static-discharge pad or (B) a laboratory bench top.

at 405 nm (OD_{405}) was determined.

The results in Figure 1 compare the OD values from individual wells of the two plates prepared either on a static-discharge pad or on a laboratory bench top. The static-discharge plate shows consistent values of OD_{405} throughout the plate. In contrast, the laboratory bench-top plate shows a wide variation in OD_{405} (range 0.4–2.4). Further, this variability is both geographic and statistically significant. The mean OD of 405 nm, from wells in columns labeled 2–8 vs. 9–11, are significantly different at $P = 3 \times 10^{-7}$ for analysis of variance (ANOVA). In contrast, the wells of the static-discharge plate, similarly analyzed, are significantly different at $P = 0.03$.

Other operators in our laboratory observed similar ELISA plate variability. Their systems used antigens of various molecular weights and pI's and were probed using several different antibodies. After using a static-discharge pad, there has been no evidence of intra-plate variability in any system (data not shown).

We did not determine what step(s) in the ELISA plate preparation were affected by static electricity. Statically charged wells might have altered the binding efficiency of a charged antigen. Alternatively, antibody binding to a sta-

tically charged antigen might have been altered. For example, Graves (1) showed that rabbit IgG nonspecifically binds to positively charged, immobilized peptides.

In summary, we present evidence that ELISA plates prepared on a static-discharge pad have lower variability as compared with plates prepared on a laboratory bench top.

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