Using the Canon PR610-2 to Develop Assays for Biowarfare Agents

Research International is actively involved in the development and manufacture of portable detection systems for counter-terrorism applications. In two of these portable detection systems, the RAPTOR™ and BioHawk™, optical waveguide sensors similar to those in the Canon system are integrated into disposable assay coupons (*see* Figure 1). When these credit card-sized assay coupons are inserted into their respective instruments, connections are automatically made to microprocessor-controlled fluidic systems that are used to perform multi-step wet assays. These assay protocols can be easily emulated with the Canon system and it has been a useful development and qualification tool for evaluating candidate antibodies, fluorophors and surface preparation techniques.

In one such protocol developed for the Canon system, the optical waveguides are picked up and moved between four positions. In Step 1 of the assay, a streptavidin coated optical fiber is incubated for 5 minutes in a biotinylated capture antibody and then moved to a wash-and-optical-read position to determine background fluorescence levels. In a typical case the waveguide might be washed for 15 seconds and read for 20 seconds. In step 2 the fiber is moved to a fluorophor-labeled antibody position for 5 min and then back to the wash-and-optical-read position to define the background signal attributable to non-specific binding of tagged antibody. In step 3 the fiber is moved to an antigen-containing sample position for 15 min to allow antibodies on the waveguide surface to bind with the targeted antigen. The fiber is then moved to the wash-and-read position to measure any change in background fluorescence. In step 4, the fiber is moved back to the labeled antibody position for 5 min and then back to the wash-and-optical-read position to measure the increase in fluorescence attributable to antigen capture by the waveguide's monolayer of bound antibody. The results are expressed as the amount of fluorescence from Step 4 minus the non-specific binding fluorescence in Step 2. The Canon system allows 4 different samples and optical fibers to be processed in one batch. Table 1 summarizes this protocol.

Using this protocol, the assay sensitivity and linearity of waveguide sensors can be determined simultaneously for up to four biothreat simulants. Typical results are shown in Table 2 and Figure 2.

Table 1: Canon Immunoassay Protocol

Assay Step	Position #	Contents	Time
1	1	Biotin-Antibody	5 min
2,4	2	Fluorophor-Antibody	5 min
3	3	Sample Antigen	15 min
1,2,3,4	4	Wash buffer	15 sec/20 sec

Table 2: Canon Optical Waveguide Immunoassay Results			
		At 2. SD	
Simulant	**Rsqd linearity**	**Calculated Det. Limit**	**Units**
Bacillus globigii spores	0.980	2.7E+04	Spores/ml
Ovalbumin protein	0.983	7.4	ng/ml

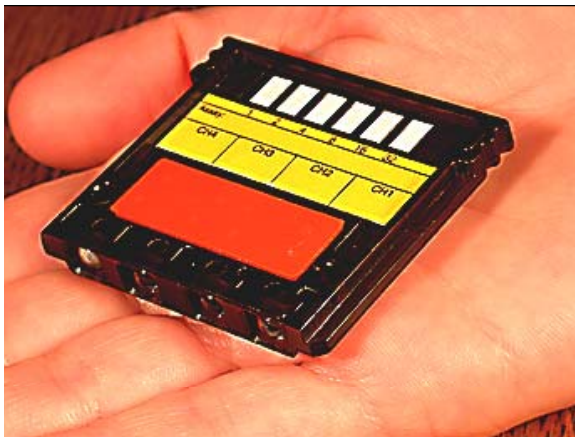

Figure 1: RAPTOR™ Bioassay Coupon.

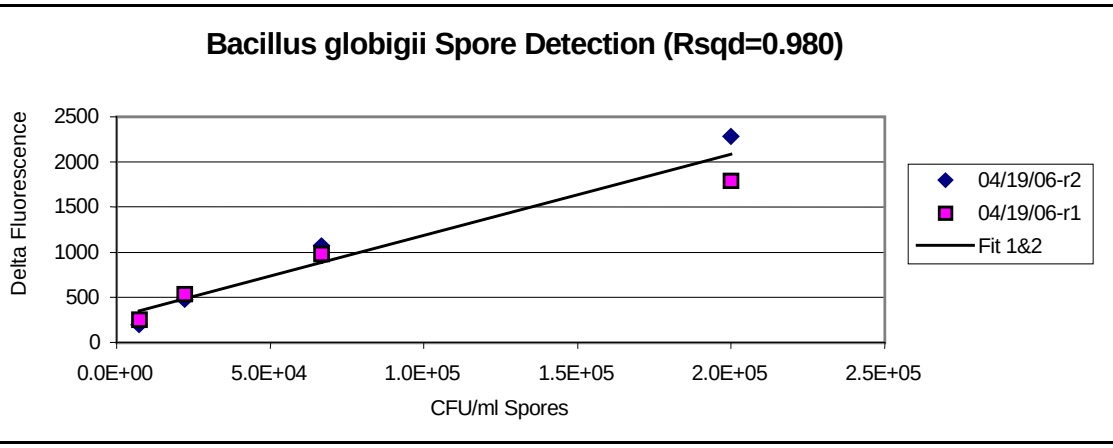

Figure 2: Bacillus globigii Spore Detection.