RAPTOR™

A Rapid, Automatic, and Portable Fluorometer Assay System

TRADEWAYS LTD

DESCRIPTION

The RAPTOR™ is a portable, 4-channel fluorometric assay system that can be used for high-sensitivity monitoring of biological agents, toxins, explosives, and chemical contaminants. It represents a careful integration of optics, fluidics, electronics, and software into one compact system for use in laboratory settings and field assays. This unit can automatically perform a user-defined, multi-step, assay protocol while simultaneously tracking fluorescently-tagged chemical reactions occurring on the surface of each of the system's four disposable optical waveguide sensors.

Using immunoassay techniques, toxins and markers such as *Y. pestis* F1 antigen have been detected at levels below 1 ppb from samples of a few hundred microliters. Each waveguide may be functionalized with a different assay, allowing up to four different assays to be run simultaneously. The results of these assays are displayed on a four line x 16 character LCD. The RAPTOR can also be run from a desktop PC, via an RS-232 link, using Windows-based software that is provided with the system.

FEATURES

The RAPTOR features an injection-molded disposable coupon containing four injection-molded optical waveguides (Patent No. 6,136,611). These waveguides are functionalized with the desired chemistry and then inserted into the coupon. They are then simultaneously interrogated using 635 nm light while monitoring the return fluorescent signal. To run an assay, the user simply inserts a coupon and presses the Run Assay key.

All of the fluidics have been automated using computer-controlled miniature valves. The system is equipped with its own internal supply of buffer, a waste bag, and constant temperature reagent storage. It supports the re-use of reagent to minimize consumption of costly immuno-chemistries; and battery power allows for up to eight hours of continuous field operation.

Assay data is automatically stored in the system's 1 MB nonvolatile EEPROM with a date and time stamp, and can be downloaded to a desktop PC using an RS-232 link. Assays typically take between 2 to 12 minutes and the results are displayed on the four line x 16 character LCD for each of the four waveguides. Additionally, a voice chip provides audio messages that can be transferred over any radio or telephone to a remote-monitoring site.

The Windows-based software allows the user to graphically monitor data recovery while an assay is running. It also provides the user with the ability to customize the various steps involved in running an assay, such as incubation times, buffer flush protocols, or the amount of data to be taken. These assay 'recipes' can be transferred, by the software, to nonvolatile memory in the RAPTOR. Upon insertion of a coupon into the instrument, an optical bar code on the coupon is automatically interrogated for recipe information. Up to 63 different assay recipes can be stored in the RAPTOR at one time.

A water-resistant, foam-lined carrying case is provided with the RAPTOR for easy transport. A zipper on top of the case allows it to be folded back, revealing a storage area for extra coupons and syringes. It is a system designed for ultimate portability; assays can be run even while the instrument is being carried.

An auxiliary electronic port is provided for controlling accessories. For example, this port can be used to interface with a Research International cyclone air sampler, the SASS 2000™, that allows airborne particulates to be concentrated in a buffer solution. Liquid levels in the sampler are maintained by microcontroller-driven electronics, and samples are automatically transferred to the RAPTOR when it is ready to run an assay. The sampler draws power from its own battery and comes equipped with a supply of buffer and a waste storage bag. The light weight and compact size of the sampler (2.8 kg w/o battery; 14.2 cm W x 19.8 cm D x 30.5 cm H) makes it easily transportable as well.

DESIGN

Internally, the RAPTOR uses a mother board/daughter card strategy allowing six different printed circuit boards to be connected together. The operation of these boards is controlled by an Intel™ 386 SX 33 MHz processor, the operating code of which is stored in nonvolatile flash RAM which allows the operating software to be upgraded easily by the user in the field.

Excitation light is provided by four 635 nm laser diodes which are electronically chopped at a frequency of 135 Hz to provide AC optical signals analogous to those produced by a mechanical chopper. Each laser is coupled to its injection-molded waveguide using a novel excitation light injection technique that incorporates a molded aspherical dielectric mirror (patent pending). Fluorescent signal light from each channel is gathered using a high efficiency lens system that collects essentially all light captured by the sensor waveguide; and a high-performance dichroic filter is used to separate excitation light from the fluorescent signal. All optics are located in one compact aluminum module with a single entrance/exit window, making it possible to quickly and easily clean the optics.

SPECIFICATIONS

Characteristic	Description
Use Profile	Indoor/outdoor sample collection, transfer, and assay; storage of 63 assay recipes; user in full MOPP gear either walking or in a slowly moving vehicle.
Physical Size	18.6 cm L x 27.4 cm H x 17.3 cm W
Weight	5.6 kg (w/o battery)
Operating Temp. Range	1 to 35°C
Storage Range	-29 to 66°C
Assay Coupon	Four simultaneous assays, disposable, coded for assay identification. Coupon reseals upon removal for archival storage.
Fluids Handling	All fluids manipulated pneumatically; sample may be oscillated to lower assay time; reagent recovered.
Fluids Storage	On-board storage of buffer and reagent. Reagent stored at constant temperature in reusable thermal storage module.
Sensitivity	Dependent on analyte, 1 to 10 ppb typical.
Photocurrent Resolution	0.019 pA; 12-bit A/D
Dynamic Range	22,500 pA
Assay Time	Dependent on assay, 7 to 12 minutes typical.
Data/Command Entry	Day/night visible keypad and display, usable in MOPP gear.
Visual	Liquid crystal display provides positive/negative/retest for each agent.
Communication	RS-232 bi-directional serial link and DB-15 accessory connector.
Data Storage	EEPROM retains raw or processed data for 200 assays.
Batteries	Primary battery BA-5590/U, 1.05 kg (2.3 lb); lifetime 9 to 24 hours.
Power Consumption	1 W, nominal
Survivability	MILSPEC 810-E
Humidity	20 to 90%, noncondensing.
Ancillary Equipment	Nylon twill photographer's-style carrying case, carry strap compatible with MOPP gear; weight 1.2 kg (2.6 lb); lump-in-cord power supply.
Accessories Support	Three digital input lines and six software-controlled external drivers.

Research International reserves the right to change specifications on any of the devices in this data sheet without prior notice.

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RAPTOR

Portable, Multianalyte Bioassay System

This rapid, automatic fluorometric assay system is a portable (6.45 kg) 4-channel system for monitoring toxins, viruses, bacteria, spores, fungi and other diverse targets. An extremely reliable third-generation product introduced in 2000, users have found these instruments will operate for two years or more with no breakdowns or leaks, and that they will tolerate debris-laden samples (such as are produced in mailrooms and food processing facilities) - impressive feats for a fully automated wet assay system.

The completely self-contained instrument is the culmination of a careful integration of optics, fluidics, electronics, and software into one compact system for laboratory and field assays. It performs user-defined, multi-step, assay protocols for monitoring fluorescently-labeled chemical reactions occurring on the surface of each of the system's four disposable optical waveguide sensors. Toxins and bacteria such as ricin and B. anthracis have been detected at levels below <1.0 ng/ml and 100 CFU/ml, respectively. **Disposable assay coupons for these and many other targets are now being offered for sale.**

The following table presents sandwich assay sensitivity data gathered from researchers worldwide who have used our equipment with a variety of raw fluid samples. Sensitivity levels may vary significantly depending on many factors, and the table is presented for reference purposes only. Please refer to the attached bibliography for further information on waveguide processing and sampling methods, or contact Research International.

Sandwich Assays Performed with Research International's Existing Bioassay Platform

Target Agent	Liquid Media	Approximate Detection Limit
Cocaine	Urine	50 ng/ml
TNT	Water	440 ng/ml
RDX	Water	1,000 ng/ml
Ovalbumin	Water	5 ng/ml
Ricin	Water	<0.5 ng/ml
Staphylococcal enterotoxin B	Water	0.1 - 0.5 ng/ml
Cholera toxin	Water	0.1 - 1 ng/ml
D-dimer	Blood plasma	200 ng/ml
Protein C	Blood plasma	160 ng/ml
Bacillus globigii	Water	2.5×10^4 CFU/ml
Bacillus anthracis	Water	30 CFU/ml
Sterne strain, vegetative cells	Whole blood	100 CFU/ml
Ames strain, irradiated spores	Water	10^4 - 10^5 CFU/ml
Erwinia herbicola	Water	10^7 CFU/ml
Yersinia pestis F1 antigen	Water	1-5 ng/ml
Brucella abortus	Water	7×10^4 CFU/ml

Francisella tularensis	Water	5×10^4 CFU/ml
Escherichia coli O157:H7	Hamburger slurry	100-1000 CFU/g (direct)
Escherichia coli O157:H7	Hamburger slurry	0.08-0.4 CFU/g (6 hour enrichment)
Escherichia coli O157:H7	Raw sewage	1000 CFU/ml
Salmonella typhimurium	Water	20,000 CFU/ml
Giardia lamblia	Drinking water	5×10^4 /ml
MS2	Water	10^9 pfu/ml
RSV	Water	Equivalent to standard ELISA

How It Works

Research International's biosensor systems are based on monolayer receptor-ligand reactions taking place on the surface of injection molded polystyrene waveguides. All fluidic and optoelectronic steps associated with the assay are performed automatically. The baseline protocol used to identify specific pathogens is the 'sandwich format' fluoroimmunoassay. In a typical waveguide-based sandwich immunoassay, the cylindrical waveguide has a monolayer of capture antibody immobilized on its surface as shown in Figure 1. Such factory-coated waveguides will maintain antibody activity for a period of months if stabilized and not subjected to high temperatures.

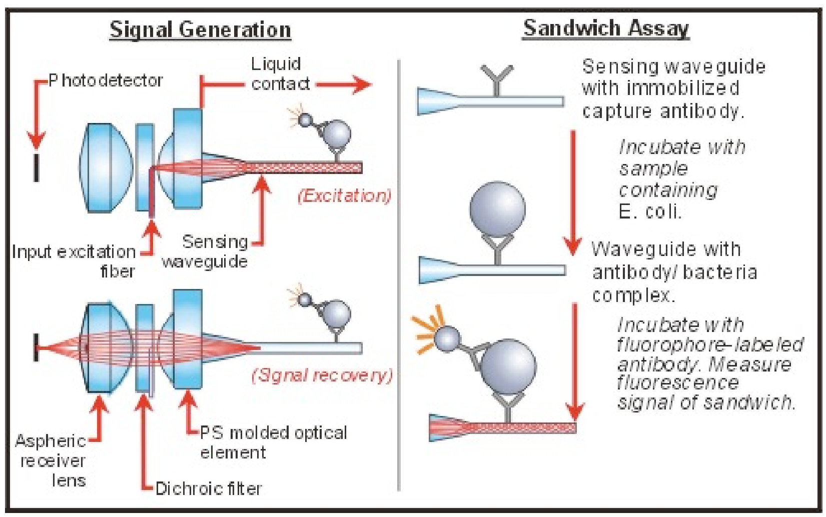

Figure 1: Optical and biochemical processes associated with a waveguide bioassay.

At the time of use, the waveguide is first incubated with the fluid sample for three to five minutes. After a wash step, the waveguide is incubated with fluorophore-labeled antibody for three to five minutes to form an antibody/antigen/labeled-antibody sandwich. These molecular sandwiches generate an optical signal when excitation light is injected into the waveguide. The excitation light creates an electromagnetic 'skin effect' in adjacent fluid and it is this so-called evanescent wave electric field that excites bound reporter molecules to fluoresce. As a final step, individual molecular signals are collectively coupled into the waveguide and monitored with a sensitive photodetector that looks down the waveguide axis.

A major problem with evanescent-excited fluoroimmunoassays has been low excitation efficiency. Light injected into a waveguide is most effective at exciting surface-bound fluorophores if the light's propagation angle is near the condition of total internal reflection (TIR) at the waveguide surface. Research International has discovered and patented a novel aspheric dielectric structure that injects light into the waveguide in such a way that evanescent electric field intensities are at near-theoretical limits. This structure, the waveguide and a signal collection lens are molded as one component, providing a highly efficient, low cost, and compact optical sensor element.

Coupons

Four of the waveguide sensors are mounted in a disposable plastic coupon (see Figure 2), allowing four different pathogens to be detected in a sample; or multiple channels may target the same pathogen to improve statistical certainty. An elastomer needle septum connects RAPTOR™ fluidics with fluid distribution channels molded into the coupon's body. In addition to providing controlled flow over the optical detectors, an assay recipe identification system is incorporated that automatically reads a coupon bar code when the coupon is mounted into the RAPTOR™ instrument. This bar code identifies the type of assay to be run by the instrument and allows very sophisticated assays to be performed by unskilled persons. A computer embedded within the RAPTOR™ performs and controls all steps in the assay procedure.

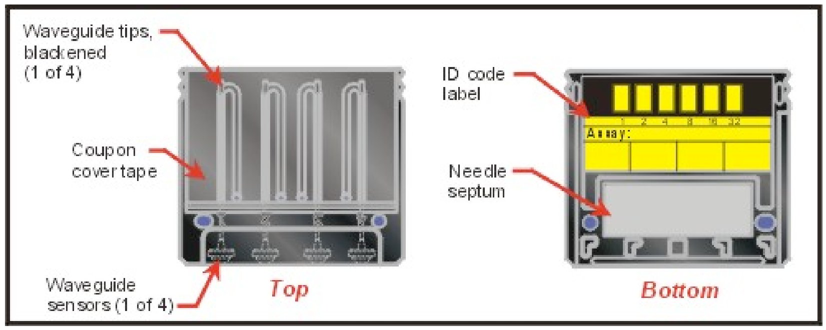

Figure 2: RAPTOR™ bioassay coupon.

Assay Procedure/Specifications

To begin an assay, a coupon is inserted into a docking bay in the instrument's top surface. Raptor and coupon optics and fluidics are automatically connected when the compartment's door is closed. The user performs an assay by turning the instrument on and pressing the Run button. During the assay, a timer in the LCD display window provides time remaining until completion. On completion, assay results for the four channels are displayed.

Behind the scenes, an onboard computer reads the recipe code on the coupon, primes the coupon for flow and controls fluidics and optoelectronic steps during the assay. All fluids needed to perform an assay, with the exception of sample, are contained in the unit. Buffer and reagent are contained in flexible on-board pressurized bags, and waste in a third unpressurized bag. The reagent bag is housed in a phase-change module that keeps the reagent at a temperature of 30°C or less, preventing deterioration of any thermally sensitive reagents.

Table 1 is a summary of system specifications. The instrument package provides a backlit six-key keypad and a four-line LCD readout. The unit can talk, control, or be controlled by other instrumentation via an RS-232 channel or RF links accessed through a connector in the instrument's battery compartment. A second connector that can supply DC power to and digitally control ancillary electronics is also provided. The unit incorporates a one-megabyte nonvolatile flash memory that can store a large number of assay protocols as well as a step-by-step log of each assay performed. An on-board BA-5590/U battery provides power for 9 to 24 hours of continuous operation depending on the backlighting intensity selected. With the battery installed, overall unit weight is about 14.2 lbs (6.45 kg), and the unit's size is 11.0" W x 7.95" H x 7.29" D.

Table 1: RAPTOR™ System Specifications

Characteristic	Description
Use Profile	Indoor/outdoor sample collection, transfer, and assay; storage of 63 assay recipes; user in full MOPP gear either walking or in a slow-moving vehicle.
Physical Size	27.9 cm W x 20.2 cm H x 18.5 cm D.

Weight	6.45 kg with battery, 5.6 kg without battery.
Operating Temperature Range	1 to 35°C
Storage Range	-29 to 66° C
Assay Coupon	Four simultaneous assays; disposable; bar coded for assay identification.
Fluids Handling	All fluids manipulated pneumatically; sample may be oscillated to lower assay time; reagent recovered.
Fluids Storage	On-board storage of buffer and reagent; reagent stored at constant temperature in removable thermal storage module.
Sensitivity	Dependent on analyte; 1 – 10 ppb typical.
Photocurrent Resolution	0.02 pA; 12-bit A/D.
Dynamic Range	$1{:}10^6$ (0.02 to 22,500 pA)
Assay Time	10– 15 minutes.
Data/Command Entry	Day/night visible keypad and display; usable in MOPP gear.
Visual/Audible Output	Liquid crystal display provides positive/negative/retest; identity of agent.
Communication	RS-232 bidirectional serial link and RF telemetry capability via BioLink RF data radio.
Data Storage	EEPROM retains raw or processed data for 100 assays.
Batteries	8-hr continuous use; primary battery BA-5590/U, 1.05 kg.
Power Consumption	7.2 W, nominal
Survivability	MILSPEC 810-E
Humidity	20 – 90%, noncondensing.
Ancillary Equipment	Nylon twill photographer's-style carrying case with storage for assay accessories; carry strap compatible with MOPP gear.
Accessories Support	Software control of **SASS 2000**™ air sampler for sample collection and sample input.

Features and Benefits

Waveguide-based fluoroimmunoassays have many features to recommend them. Although similar in some ways to traditional ELISA protocols, they are much faster and comparable in sensitivity: Typical assay times of 10-15 minutes are possible because pathogen targets are reacting with exposed monolayer-thick reaction layers. Since waveguide capture layers target a single or very narrow range of pathogens, sensor coupons may be reused if test results continue to be negative. This may very favorably lower cost per assay, since in many survey applications most assays will be negative.

In a lab setting, assay sensitivity was unaffected by 30 consecutive negative assays performed using clean samples. While field results may vary, the waveguide sensors are remarkably insensitive to sample contamination and have been successfully used with urine, whole blood, milk, marine water, 10% meat slurries and slurries of human waste. In addition, bacteria captured on the waveguide surface are still viable, and the system can serve as an affinity capture surface for later confirmatory analyses and chain-of-custody needs.

The Raptor fiber optic biosensor system represents a significant leap in technology that is capable of real time detection of microbial pathogens with or without conventional culture. Furthermore, the biosensor is a compact, portable system that can easily be adapted with flow-through devices for on-line monitoring of water systems. This technology can also be adapted to quantitation of all microorganisms against which a specific antibody can be made. As new pathogens emerge the biosensor can be adapted to signal their presence. Unlike many recent developments in microorganism detection, which represent minor improvements over current technology, an immunoassay-based biosensor for real time or near real time detection of microbial pathogens represents a pivotal advance in scientific technology.

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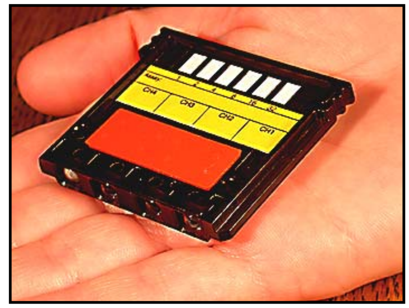
RAPTOR™ Assay Coupons



The RAPTOR™'s disposable bioassay coupon incorporates four polystyrene waveguide biosensors sealed into a credit card-size injection molded polycarbonate shell (See Figure 1). The shell houses the waveguides, a series of flow channels to direct fluids through the coupon, and a resealable needle septum that is used to connect fluids in the RAPTOR™ to the coupon channels.

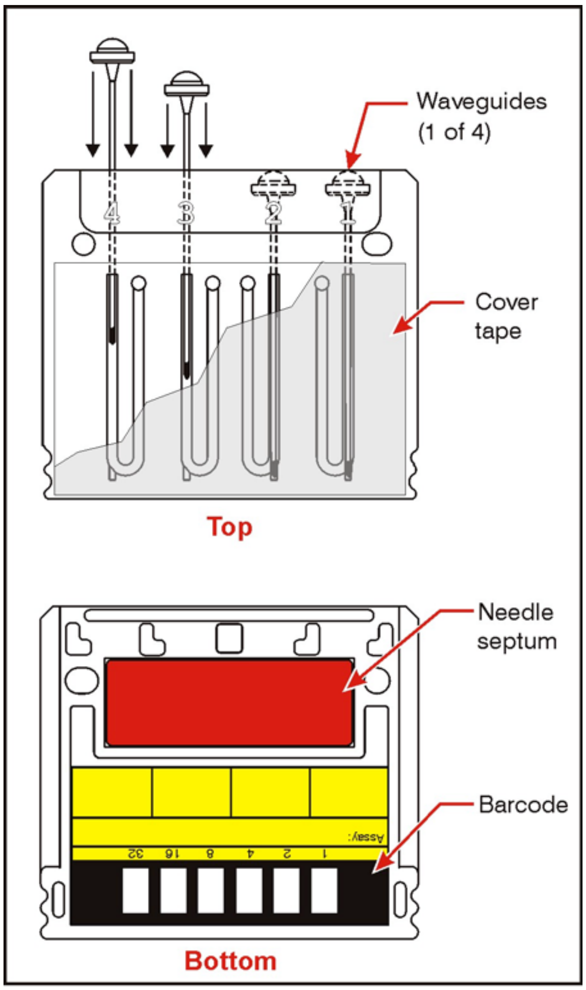


Figure 1: Key features of RAPTOR™ bioassay coupon.

Each coupon is also supplied with four 1cc vials of reuseable reagent (in freeze-dried form) that are automatically re-hydrated by the RAPTOR™ fluidics before first being used in the assay process. The liquefied reagents are stored in these vials within a temperature-controlled compartment in the RAPTOR™ when not needed. Many research-oriented users mix these reagents together into one 'cocktail," but there is a small risk that some reagents may interact: Research International has taken the conservative approach of providing the architecture needed to separately store and dispense them.

In addition to providing controlled flow over the sensor elements, the coupon incorporates an assay recipe identification system in the form of a bar code that is automatically read when the coupon is inserted into the instrument. This bar code identifies the type of assay to be run and allows up to 62 different and very sophisticated multi-step assays to be performed by untrained persons, since a microprocessor within the instrument performs and controls all assay procedure steps.

For the results-oriented user, pre-assembled coupons are available and Research International will also provide custom coupons for specific analytes on a quotation basis. All coupon components are available for sale separately for the hands-on researcher, and the RAPTOR™ manual provides detailed information on how to coat waveguides and assemble coupons.

The Tables on the back of this page provide information on analytes that have been successfully detected using the RAPTOR™ technology, as well as general coupon specifications.

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Table 1: Analyte Target Sheet(1)		
Pathogen	**Description/Media**	**Limit of Detection**
Ricin	Protein toxin / Water	1 ng/mL
Staphylococcal enterotoxin B	Protein toxin / Water	0.1 – 0.5 ng/mL
Bacillus anthracis (vegetative Sterne cells)	Vegetative cells / Whole blood	100 CFU/mL
Bacillus anthracis (irradiated Ames spores)	Irradiated spores / Water	5 x 10^4 CFU/mL
Escherichia coli O157:H7	Vegetative cells / Hamburger slurry	100 – 1000 CFU/mL
Giardia lamblia	Vegetative cells / Drinking water	5 x 10^4 CFU/mL
Yersinia pestis F1 antigen	Capsular protein from cell wall / Water	1 ng/mL
Ovalbumin	Protein toxin simulant / Water	5 ng/mL
Cholera toxin	Protein toxin / Water	0.1 – 1 ng/mL
Protein C	Blood component / Blood Plasma	160 ng/ml
Bacillus globigii	Sporulated bacteria simulant / Water	2.5 x 10^4 CFU/mL
Brucella abortus	Vegetative cells / Water	7 x 10^4 CFU/mL
Francisella tularensis	Vegetative cells / Water	5 x 10^4 CFU/mL
Salmonella typhimurium	Vegetative cells / Water	2 x 10^4 CFU/mL
Vaccinia virus	Pox virus / Water	10^5 PFU/mL
Cocaine	Drug of abuse / Urine	50 ng/ml
TNT	Explosive / Water	440 ng/ml

Table 2: General Coupon Specifications(2)	
Parameter	**Value**
Assay method	Antibody-based sandwich bioassay on the surface of a disposable plastic waveguide.
Number of pathogens monitored in a single coupon	4 waveguide sensors/coupon. Each is specific for a single pathogen. The factory-installed sensors may be for 4 different pathogens, or some pathogens may be repeated to improve statistical certainty.
Assay time	10 to 15 minutes
False positive rate	1.25 – 2.5% per waveguide. If multiple waveguides are used in the same coupon the false positive rate decreases as the product of the single waveguide rate.
Number of times a coupon can be reused	Minimal degradation after 30 negative assays. Coupons should be replaced if a strong positive response is registered.
Maximum operating time	8 to 12 hours typical if all coupon assays have been negative.
Maximum use temperature	Antibodies rapidly degrade above 40° C
Storage recommendations	Refrigeration at 4° C provides longest shelf life.
Shelf life	Research grade(3): 1 month, typical at 20° C Production qualified: 3 to 6 months typical at 20° C
Reagents	Each coupon is supplied with 4ea. 1 cc vials of freeze-dried fluorescent reagent that are installed in the RAPTOR.
General precautions	Keep coupons out of strong sunlight or high temperatures. Do not damage inset lenses at the coupon's thicker end.

(1) RAPTOR System bioassays performed by various research groups. Production-qualified assays can be expected to exhibit similar or better sensitivity. RI does not warranty the accuracy of measurements made by these third-party researchers or that the protocols used are practical for all field applications. This data is for informational purposes only.
(2) Certain specifications such as shelf life and thermal stability are not expected to vary substantially from one pathogen to another. These generic Specifications are presented for antibody-based coupons used in a sandwich assay format.
(3) Research grade coupons are made using small-lot lab bench methods and have not been subjected to the same level of quality control and assay optimization as production-quality coupons.