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CONTRACT NUMBER DAMD17-96-C-6038

TITLE: In Field Detection of Biologicals in Human Blood Serum,
Saliva and Urine Using Pan Coated Quartz Crystals

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REPORT DATE: October 1996

TYPE OF REPORT: Final, Phase I

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

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REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE October 1996	3. REPORT TYPE AND DATES COVERED Final, Phase I (1 Mar 96 - 31 Aug 96)	
4. TITLE AND SUBTITLE In Field Detection of Biologicals in Human Blood Serum, Saliva and Urine Using Pan Coated Quartz Crystals		5. FUNDING NUMBERS DAMD17-96-C-6038	
6. AUTHOR(S) Robert Carter			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Universal Sensors, Inc. Metairie, Louisiana 70006		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, MD 21702-5012		10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES		19961120 006	
12a. DISTRIBUTION / AVAILABILITY STATEMENT Distribution authorized to DOD Components only (specific authority). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) Quartz crystal microbalance biosensors to detect ricin in complex biological matrices such as serum, saliva and urine have been developed. The sensors utilize a novel polyacrylonitrile (PAN) or protein G coating which is insensitive to non-specific fouling. The biosensor relies on simple interaction of immobilized anti-ricin antibody against the free antigen in solution. As antigen attaches to immobilized antibody, a frequency change is detected due to the additional mass on the piezoelectric crystals surface. The sensors will detect nanogram per milliliter amounts of ricin from these matrices with little or no sample pre-treatment or dilution. The biosensor assay is fast with results possible in approximately 500 seconds. The use of biosensor-based assays, in-field, may expedite clinical analysis and triage for exposure to possible biological warfare agents.			
14. SUBJECT TERMS SBIR, Human Blood Serum, Urine, Saliva, Quartz Crystals, Protein G Coating		15. NUMBER OF PAGES 38	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Limited

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Robert M. Park
PI - Signature

9/17/96

Date

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1 Introduction: Identification and significance of the problem

The ultimate goal of this research effort was to develop biological sensors for the detection of ricin in complex matrices such as serum, saliva or urine. The prepared biosensors are based on the use of quartz crystal microbalance techniques which utilize immunochemical reactions for the detection of ricin. These reactions occur on the transducer's active portion of the biosensor, which is sensitive to changes in mass and detected as change in frequency. As the mass to frequency ratio is proportional, there exists a well defined relationship of the analytes mass specifically attaching to the transducer, which is noted by a change in frequency. The use of quartz crystal microbalance techniques for detection of biologicals has several advantages: 1) signals generated by analyte detection are not affected by dirty matrices, 2) they do not require sample pretreatment for analysis, and 3) assays do not require a secondary label for creation of the detectable analytical signal.

At present, individual combatants have no way of knowing if they or their environment are contaminated with chemical warfare (CW) or biological (BW) agents. Therefore, they are encumbered by heavy protective suits if there is a reasonable chance that BW agents will be deployed or have contaminated an area. These suits greatly reduce the efficiency of the soldiers as they are heavy, uncomfortable and reduce perceptions of touch, sight and sound. In addition, they can engender claustrophobia, apprehension and panic.¹ Non compliance, due to these problems, increases the chance that bodily contamination could occur. The need then arises for rapid detection of the BW agent in a biological matrix, such as serum, as well as detection of circulating antibody to these agents. Due to these facts, rapid detection of either antigen or antibody, with simplified equipment, becomes a necessity.

With the general increase in the possibility of biological warfare agents being used on soldiers or civilians, the need for a strong deterrent gains significance. The better the protection against attacks of this nature, the greater the incentive to decrease the likelihood of their use by hostile forces. Documents of World War II indicated that when both sides of the war knew the opponent was properly equipped, neither side used chemical weapons. Not since the early days of World War I, where chlorine gas clouds were first used by the Germans, has there been such renewed interest in protection from chemical or biological weapons and their effects on humans. Historically, CW or BW agents have been used only on forces lacking protection, for example, Ethiopia (1935), China (1938), Yemen (1966), and more recently in the Middle East and Japan.

The ability to rapidly assay for antigens or antibodies, in real-time, would benefit both the ARMY and civilians. The production of a fast, inexpensive, reliable device which could perform both types of assays would increase the safety factor for unsuspecting personnel and may become a significant tool in clinical settings.

Roederer and Baastians² used PZ devices coated with antibody to determine antigen in solution. This type of assay has been expanded upon for the detection of specific bacteria by Plomer, Guilbault and Hock³, Prusak-Sochaczewski,⁴ and Jacobs,⁵ et al. Viruses have also been detected by similar PZ methods. Konig and Gratzel⁶ described PZ detection of herpes virus in serum using

PZ crystals coated with specific antibody and instrumentation developed by Universal Sensors, Inc. Ebersole⁷ has described detection of Herpes Simplex virus nucleic acids in serum. Other investigators, for example Yamaguchi⁸, have used PZ methods to determine adsorption and hybridization of DNA in solution. An overview of this technology is described further by the President of Universal Sensors.⁹ Additional information on the use of quartz crystal microbalances for immunoassay will be reported in an upcoming review article. This article features descriptions of available hardware for performing these types of analysis.¹⁰ This product review includes Universal Sensors, Inc's PZ1000 Immunobiosensor Detector which was used in this study. In addition, discussions of the current PZ technologies are described, indicating where the devices are used or will be used in the near future.

1.1 Use of toxins as biological warfare agents

Organisms or plants which produce toxins that are used for military purposes are classified as chemical warfare agents (CWA). Due to the recent advances in genetic engineering, synthetic toxin molecules are capable of being batch produced. This large volume of toxin accelerates the possible production of CWA and their inevitable use. Toxins which are not available through fermentation (bacterial), plant sources (ricin) or through genetic engineering can, in reality, only be used for small scale operations such as sabotage.

Isolated from the castor oil plant, ricin was patented for use in chemical weapons by the U.S.A. in 1953. Ricinus sp., due to its wide geographical distribution, is capable of cultivation in most temperate to semi-tropical areas. This hardy fast growing plant produces prolific flowers which produce poisonous seeds. Humans or animals that ingest these seeds, after mastication, will experience severe gastrointestinal upset with sensory motor skills involvement. This type of toxin is easily produced on a large scale through cultivation and can be impregnated onto projectiles for injection into military personnel. This toxin also can be dusted onto an area where it will be tracked onto and distributed by military hardware and unsuspecting military or civilian personnel.

During the 1970's, ricin was used as a chemical weapon in the Bulgarian "Umbrella Murders". A special shooting "umbrella", which propelled ricin laced bullets was able to kill humans after 24 hours of excruciating debilitation.¹¹ Protection from ricin toxicity has recently been demonstrated by vaccination or administration of passive heterologous antibody in mice.¹² An ELISA to detect ricin has been described by both Carter and Poli using antibodies manufactured by USAMRIID personnel.^{13,14} In addition, Carter has reported that ricin and its antibody have been detected in buffered saline solutions with a piezoelectric format.¹⁵ It was unknown how the described sensors would operate in more complex biological matrices. The drive to identify the presence of biological agents in these matrices forced the design of new quantitative methods for their detection. The previously developed piezoelectric detections offered sufficient quantitation of ricin to warrant further investigation into evaluation of more complex biological matrices.

1.2 Piezoelectric theory

In the PZ format, a piezoelectric transducer, which consists of a quartz crystal with two coated

gold electrodes, connected to an oscillator, will detect the attachment of antibody to immobilized antigen or the attachment of antigen to immobilized antibody on the surface of the transducer. As mass increases on the surface of the transducer, frequencies will decrease in an inversely proportional manner. A frequency-to-mass ratio of the bound antibody or antigen can be determined by the Sauerbrey equation:¹⁶

$$\Delta F = -(2.3 \times 10^{-6})F^2 \Delta M/A$$

where ΔF is the fundamental frequency change, F is the resonant frequency, ΔM is the change in the mass deposited on the crystal and A is the area of the quartz crystal which is coated. As antibody or antigen specifically attaches to the PZ transducer, an inversely proportional frequency change will occur as mass increases. Piezoelectric technologies are not adversely affected by turbidity, color and mixed medias. This makes PZ a selective method for detection, in-field, where other methods may be subject to fouling or interference from the complex matrices.

Another advantage to PZ determination of antibody or antigen is that no sample pre-treatment is needed before analysis, which shortens overall assay time. Piezoelectric determinations also have the advantage of not requiring a secondary label such as radioactivity or enzyme to produce an analytical signal. This advantage is only shared with surface plasmon resonance (SPR) devices. However, unlike SPR, PZ determinations do not require expensive instrumentation, sample pre-treatment and highly trained personnel. SPR and PZ detection have been favorably compared and display similar detection limits.¹⁷

1.3 New polyacrylonitrile (PAN) support matrices for biosensors

The desire to detect biological toxins in complex matrices such as serum, saliva and blood is of high priority to clinicians as well as ARMY personnel. We have successfully created a piezoelectric detection format which can identify ricin in these matrices. The biosensors are specific, require no additional reagents and use minimal sample pretreatment prior to analysis. These biosensors were created to utilize polyacrylonitrile (PAN), which is a novel coating for immobilizing capture antibody on the transducers surface. PAN coatings were originally developed by Sandia National Laboratories, Albuquerque, NM. Their laboratories have collaborated with other outside agencies and post developers for PANs inclusion into various devices including piezoelectric-based biosensors.

PAN, due to its unique properties, can be applied either as a dense matrix or as a foam cell matrix. Advantages of using the foam cell are due to its increased surface area for attachment of capture antibody. A ten to twenty fold increase in area is noted versus dense coatings such as protein A, protein G, or dense PAN.

PAN matrices allow for direct attachment of antibody or antigen to its surface using crosslinking reagents. This process provides antibodies with a strong support for reaction with analyte. The created biosensors utilized PAN coatings and were compared to conventional biosensor coatings such as protein A or G for detection of ricin in urine, saliva and serum. These detections were

monitored in real-time with the use of software developed in house. This software, PZTools, monitors frequency changes as they occur and depicts them graphically. In addition, the software can also derive kinetics of association or dissociation when different analyte concentrations are compared. This is a rapid way to compare unknown samples to standard binding (associations), stored prior to analysis, for quantitative measurements.

2.0 Experimental procedures and results.

2.1 ELISA

2.1.1 Development

ELISA's were developed to detect ricin in Dulbecco's phosphate buffered saline (DPBS)^a with three different capture antibodies, with alternation of the secondary antibody. These were performed to evaluate newly acquired rabbit anti-ricin against USAMRIID goat anti-ricin. (Goat anti-ricin is no longer available from USAMRIID due to its high cost. *All plates, beakers or other items which come in contact with ricin solutions are decontaminated with a 10% bleach solution prior to disposal.*

2.1.1.1 Goat IgG primary, goat IgG- biotin secondary antibody

Capture antibody, goat anti-ricin, was diluted to 50 µg / mL in DPBS, added to triplicate wells of a 96 well microtiter plate and incubated for one hour at 37°C. The plate was washed four times with 300 µL / well of phosphate buffered saline which contained 1% BSA and 0.1% tween (DBTT). Wells were then blocked with the same buffer for one hour at room temperature, washed and aspirated as described previously.

Ricin stocks were diluted in Dulbeccos phosphate buffered saline in a range of 0.5 to 8 µg / mL or 8 ng / mL to 0.1 ng / mL including blanks, 50 µL / well, and incubated for one hour at 37°C. The plates were washed as described above then aspirated. Microwells were then incubated with 50 µL / well of a 1:200 dilution of goat anti-ricin antibody labelled with biotin^b in phosphate buffered saline. The plate was incubated, washed and aspirated as described above. Microwells were then

^a DPBS consists of 137 mM NaCl, 2.7 mM KCl, 8.0 mM Na₂HPO₄ 7H₂O and 1.5 mM KH₂PO₄. pH 7.44.

^b Antibody was labeled with biotin as per manufacturers directions. Briefly, antibody was placed within a dark amber vial covered with tin foil and a small magnetic stirring rod was inserted into the solution. Biotin was added slowly to the reaction mixture, mixed and incubated for one hour at room temperature. Biotinylated antibody was recovered by centrifugation with a 30K MWCO vial which allowed free biotin to pass through while retaining antibody which is biotin labeled. Extensive rinsing with DPBS results in the removal of most free or unbound biotin.

subjected to avidin which was labeled with alkaline phosphatase, 50 μL / well, at a dilution of 1:500 in phosphate buffered saline from stock. The plates was incubated, washed and aspirated as described previously. All rows were exposed to 50 μL of 1 mg / mL paranitrophenyl phosphate (PnPP) in 50mM carbonate buffer with 0.1mg / mL MgCl_2 , pH 9.8 buffer and incubated for color development. Absorbencies were then read at 405 nm in a microplate reader. Figure 1 and 2 demonstrate detection of ricin in DPBS.

2.1.1.2 Rabbit IgG primary, goat IgG or IgG-biotin secondary antibodies

Capture antibody, Sigma or EY Laboratories rabbit anti-ricin, was diluted 1:20 from stock in phosphate buffered saline and added to triplicate wells of a 96 well microtiter plate. After incubation for one hour at 37°C, the plate was washed four times with 300 μL / well of phosphate buffered saline which contained 1% BSA and 0.1% tween (DBTT). Wells were then blocked with the same buffer for one hour at room temperature, washed and aspirated as described previously.

Ricin stocks were diluted in phosphate buffered saline in a range of 8 ng / mL to 0.1 ng / mL including blanks, 50 μL / well, and incubated for one hour at 37°C. The plates were washed as described previously then aspirated. Microwells were then incubated with either 50 μL / well, biotinylated goat anti-ricin antibody or goat anti-ricin at a 1:200 dilution of stock in phosphate buffered saline. The plate was incubated, washed and aspirated as described above. Microwells that received biotinylated were then subjected to avidin which is linked to 100 units alkaline phosphatase, 50 μL / well, at a dilution of 1:500 in phosphate buffered saline from stock. Microwells that received unlabeled goat anti-ricin were then subjected to rabbit anti-goat/alkaline phosphatase, 50 μL / well, at a dilution of 1:20,000 in phosphate buffered saline from stock. The plates were incubated, washed and aspirated as described previously. All rows were exposed to 50 μL of 1 mg / mL paranitrophenyl phosphate in 50 mM carbonate buffer with 0.1 mg / mL MgCl_2 , pH 9.8 buffer and incubated for color development. Absorbencies were then read at 405 nm in a microplate reader. Data from these experiments are depicted in Figure no. 2.

The urine and saliva-based ELISA's utilized an affinity purified goat anti-ricin antibody prepared by USAMRIID as well as two rabbit anti-ricin antibodies. The serum ELISA used the best configurations found in saliva and urine. A brief description of these procedures follows.

2.1.2 Detection in urine

2.1.2.1 Rabbit IgG capture, goat IgG-biotin secondary antibody

Microplates were labeled with Sigma capture antibody as described in section 2.1.1 Ricin stocks were diluted in urine^c in a range of 8 ng / mL to 0.1 ng / mL including blanks, 50 μL / well, and

^cHuman Urine Control, Urine consisted of 15 mL of reconstituted urine control. (Count 10-Trol 1, V-tech, Inc, Pomona, CA)

incubated for one hour at 37°C. The plates were washed as described previously then aspirated. Microwells were then incubated with 50 μL / well of a 1:50 dilution of either Sigma or EY laboratories rabbit anti-ricin antibody in phosphate buffered saline. The plate was incubated, washed and aspirated as described above. Microwells were then subjected to goat anti-rabbit / alkaline phosphatase, 50 μL / well, at a dilution of 1:20,000 in phosphate buffered saline from stock. The plates was incubated, washed and aspirated as described previously. All rows were exposed to 50 μL of 1 mg / mL paranitrophenyl phosphate in 50mM carbonate buffer with 0.1mg / mL MgCl_2 , pH 9.8 buffer and incubated for color development. Absorbencies were then read at 405 nm in a microplate reader. Data is depicted in Figure no. 3

2.1.2.2 Goat IgG capture, rabbit IgG secondary antibody

Microplates were labeled with capture antibody as described in section 2.1.1 The plate was washed four times with 300 μL well of phosphate buffered saline which contained 1% BSA and 0.1% tween (DBTT). Wells were then blocked with the same buffer for one hour at room temperature, washed and aspirated as described previously.

Ricin stocks were diluted in urine in a range of 8 ng / mL to 0.1 ng / mL including blanks, 50 μL / well, and incubated for one hour at 37°C. The plates were washed as described previously then aspirated. Microwells were then incubated with 50 μL / well, biotinylated goat anti-ricin antibody at a 1:200 dilution of stock in phosphate buffered saline. The plate was incubated, washed and aspirated as described above. Microwells were then subjected to avidin which is linked to 100 units alkaline phosphatase, 50 μL / well, at a dilution of 1:500 in phosphate buffered saline from stock. The plates was incubated, washed and aspirated as described previously. All rows were exposed to 50 μL of 1 mg / mL paranitrophenyl phosphate in 50mM carbonate buffer with 0.1mg / mL MgCl_2 , pH 9.8 buffer and incubated for color development. Absorbencies were then read at 405 nm in a microplate reader. Data is depicted in Figure no. 3

2.1.2.3 Rabbit IgG capture, goat IgG secondary antibody

Capture antibody, EY Laboratories, rabbit anti-ricin was diluted 1:20 from stock in phosphate buffered saline and added to triplicate wells of a 96 well microtiter plate. After incubation for one hour at 37°C, the plate was washed four times with 300 μL well of phosphate buffered saline which contained 1% BSA and 0.1% tween (DBTT). Wells were then blocked with the same buffer for one hour at room temperature, washed and aspirated as described previously.

Ricin stocks were diluted in urine in a range of 8 ng / mL to 0.1 ng / mL including blanks, 50 μL / well, and incubated for one hour at 37°C. The plates were washed as described previously then aspirated. Microwells were then incubated with either 50 μL / well, biotinylated goat anti-ricin antibody or goat anti-ricin at a 1:200 dilution of stock in phosphate buffered saline. The plate was incubated, washed and aspirated as described above. Microwells that received biotinylated IgG were then subjected to avidin which is linked to 100 units alkaline phosphatase, 50 μL / well, at a dilution of 1:500 in phosphate buffered saline from stock. Microwells that received unlabeled goat anti-ricin were then subjected to rabbit anti-goat/alkaline phosphatase, 50 μL / well, at a

dilution of 1:20,000 in phosphate buffered saline from stock. The plates were incubated, washed and aspirated as described previously. All rows were exposed to 50 μ L of 1 mg / mL paranitrophenyl phosphate in 50 mM carbonate buffer with 0.1 mg / mL $MgCl_2$, pH 9.8 buffer and incubated for color development. Absorbencies were then read at 405 nm in a microplate reader. Data is depicted in Figure nos. 4 and 5.

2.1.3 Detection in saliva with and without protease inhibitors

Goat capture antibody ELISA with rabbit secondary antibody was applied to the microplate as described in section 2.1.1. Ricin stocks were diluted in saliva^d in a range of 0.1 ng / mL to 100 ng / mL including blanks, 50 μ L / well, and incubated for one hour at 37°C. The plates were washed with DBT which contained 0.1% tween (DBTT) as described previously, then aspirated. Microwells were then incubated with 50 μ L / well of a 1:500 dilution of Sigma rabbit anti-ricin antibody in DBT. The plate was incubated, washed with DBTT and aspirated as described above. Microwells were then subjected to goat anti-rabbit / alkaline phosphatase, 50 μ L / well, at a dilution of 1:20,000 in DBT from stock. The plates were incubated, washed and aspirated as described previously. All rows were exposed to 100 μ L of 1 mg / mL para-nitrophenyl phosphate in 100 mM carbonate buffer with 1 mM $MgCl_2$, pH 9.8 buffer and incubated for color development. Absorbencies were then read at 405 nm in a microplate reader. Data is depicted in Figure no. 6.

Saliva was treated in two ways during collection as described below, then run in separate ELISAs. Data from each of these experiments are depicted in Figures 6 and 7, respectively.

Due to the poor response of ELISA detection of ricin in saliva, it was decided to investigate what may be responsible for the lower than expected detection levels. Most obvious problems would occur due to protease or other enzymatic activity found nacesently in human saliva. Due to this phenomena, an ELISA was developed to evaluate controls of both enzymes and proteases.

Due to lower than expected detection values for the ELISA when using saliva as the biological matrices, we attempted to improve the assay via the use of protease inhibitors. As saliva contains significant amounts of enzymatic activity, it seems plausible that they may have an inhibitory effect on the detection of ricin. If saliva components are having a detrimental effect, a significant improvement of signal and detection levels will result from inhibition of these elements. As proteases and peptidases are commonly found in saliva, EDTA and PMSF are obvious choices to

^d Saliva samples were collected within a 30 min period. Heavier debris was allowed to settle out, leaving a top clear layer which was centrifuged for 10 min at 5000 rpm at 4° C. The debris was pelleted and the clear layer was used for ricin dilutions. The collection procedure was later modified by keeping the saliva cold during all steps. The sample was then spun at an integral of 969E4 (RCF=19400g) for 3 min. The top layer was separated from particulate matter and spun again at 3000 rpm for 3 min. The supernatants were removed and stored at 4° C for use in assays.

inhibit these proteins. Other enzymes, such as α -amylase, are also found in abundance in saliva. This enzyme can also be controlled by specific inhibitors. None of these inhibitors is expected to have a direct effect on salivary ricin as it is not in the class of proteins affected by these reagents.

A microtiter plate was labeled with 50 μ L of a 50 μ g/mL stock of goat anti-ricin in DPBS. Plate was incubated for one hour at 37°C, washed three times with 300 μ L of DBT and blocked with same. Plates were aspirated and 50 μ L of saliva samples, untreated or treated with either 0.05% Tween or 5 mM EDTA, was added and incubated for one hour at 37°C. Plates were washed as described above and subjected to 1:500 dilution of rabbit anti-ricin secondary antibody, incubated for one hour and washed as described previously. Plates were then incubated with 50 μ L of 1:20,000 dilution of goat anti-rabbit IgG/Alkaline phosphatase for one hour at 37°C and washed as described above. All wells were then exposed to 100 μ L of a 1 mg/mL PNPP solution in carbonate buffer and absorbencies were recorded with a microplate reader. Figure no. 6 indicates that while addition of Tween results in a decrease in detection of ricin, treatment of saliva with EDTA allows for an increase in absorbance.

Another ELISA was conducted in which the plate was labeled with capture antibody and blocked as before, then ricin was diluted in saliva samples which were untreated or treated with either 50 μ M PMSF, 75 units α -amylase inhibitor, or 5 mM EDTA. As noted in Figure no. 7, no significant difference is noted within the four groups with the exception of EDTA treated saliva. This data is not consistent with the previous ELISA results above, which suggested an advantage when using EDTA as an salivary protease inhibitor.

2.1.4 Detection in serum

The procedure for detection of ricin in human serum^e is essentially the same as described for Dulbeccos phosphate buffered saline and urine. Goat anti-ricin antibody was used as the capture and rabbit anti-ricin (Sigma) was used as the secondary. The plates were labeled as described in section 2.1.1 However, the blocking step was changed to include either a 1% BSA block or a blocked with 1% Human serum in DPBS for one hour. All other steps were as described previously. All ricin dilutions were made in undiluted stock human serum.

As depicted in figure no. 8, Detection of ricin in these matrices is possible. To our knowledge, ELISA detection of ricin in matrices such as these have not been previously described. Even though there are subtle differences between detection levels depending on the antibody pairs utilized or the matrix used, we feel that these ELISAs are important tools for evaluation of possible ricin contamination in biological matrices. As demonstrated in figure 8, the effect of either the BSA or Human serum block was not apparent. The ELISA was able to detect 0.5ng/mL ricin in undiluted serum.

^eNormal Human Serum, Sigma Chemical Co. St. Louis.MO.

2.2 Polyacrylonitrile (PAN) system

2.2.1 Preparation of PAN coated crystals.

The unique structure of the PAN molecule allows for direct attachment of the antibody to the PAN matrix. An available binding site composed of nitrogen allows for attachment to the biomolecule (antibody) with crosslinking reagents such as ethyldimethylaminopropylcarbodiimide (EDC). This reagent attaches the carboxy terminal portion of the antibody to the nitrogen group on the PAN surface. This covalent attachment prevents leaching of the capture antibody from the transducers surface during experimentation.

The crystal's spring mounts are detached from the base mount and cleaned with acid solutions to remove any machine oils that may be present. The crystal with springs is then placed into a chuck which firmly holds the crystal in place in the proper orientation. The chuck then has a mask placed over the crystal surface which allows only the gold transducer portion of the crystal to be available for coating with PAN. The chuck is then placed into a controlled environmental chamber and humidity and temperature are adjusted for control of the PAN foam cells matrix density and porosity.

Once the chamber has been equilibrated, various percent solutions of PAN are spin cast onto the masked area of the quartz crystal using techniques which control the density of deposition. Crystals are allowed to cure for a brief period, then removed from the chamber. Crystals are then remounted onto the original holding mounts and stored until sufficient numbers are generated for shipment to Universal Sensors, Inc for labeling with appropriate capture antibody.

Later into the research project, an adaptation of the PAN coating process was developed. This is described in the following text. PAN coated crystals were received from Sandia National Laboratories which were treated with an updated PAN coating procedure. A new mask was developed which allows the crystals to be placed into the spin coating device without removal of the spring mounts from the base. Two major advantages became available by this new treatment. 1) Unsoldering and resoldering of the base can now be omitted during the coating process and 2) More reproducible coatings on the gold surface can now be obtained. This is important for both lot-to-lot control as well as the time it takes for creation of the coating procedure. The first set of experiments with these crystals is described later in this proposal.

The foam cell size can be controlled with the amount of available humidity when it is spun onto the transducer surface. Generally, when humidity is increased, there is a corresponding increase in foam cell size. Tight control of humidity during the coating process is necessary to regulate the cell size on a run-to-run basis. Due to this, only persons highly trained in the application of this polymer, such as Sandia personnel, are qualified to produce coated crystals of this type. A patent is pending on the production of PAN coated biosensors which was prepared by Universal Sensors and Sandia National Laboratories.

2.2.2 Detection in urine

2.2.2.1 Static measurements

Initial, unlabeled frequencies were recorded. On specific crystals, 5 μ L of goat anti-ricin,* 1 mg / mL, was added to the transducer surface along with 5 μ L of a 1mg / mL EDC crosslinker solution. Non-specific crystals were treated similarly to a 1mg / mL solution of goat anti-rabbit antibody. The mixtures were allowed to react at 37°C with the PAN surface for one hour in a humidified atmosphere, rinsed with phosphate buffered saline then distilled water and dried. Frequencies were measured to determine the amount of specific or non-specific capture antibody bound on the PAN surface.

Crystals are assembled in a to Universal Sensors, Inc flow cell,^f 50 microliters of phosphate buffered saline or urine was added to the cell on the static side and the crystals frequencies were allowed to equilibrate for approximately 5 minutes.

Fifty five microliters of urine containing 20 ng / mL ricin were added to the cells and frequencies were monitored using PZTools™ software^g for real-time analysis of analyte binding. One second intervals of data from both specific and non-specific crystals were recorded as frequency change versus time. No specificity was noted with these experiments. (Data not shown)

^f PZ liquid/flow cells, developed by Universal Sensors, Inc., were used for detection of ricin. This cell allows the use of liquid samples with a PZ crystal in two ways. One side of the cell is constructed as a flow system. In this case, one face of the crystal is exposed to a 70 μ L chamber, which is connected to an external peristaltic or syringe pumping system through standard tubing fittings. The other side is constructed as a static system. In this case, one face of the crystal is exposed to a chamber that can hold up to 1 mL of liquid. The quartz crystal is connected to the detector through a shielded cable.

^gThe PZTools™ program is designed to read frequency data from piezoelectric detectors. The program is organized to several specialized windows performing the following tasks:

- 1) Measurements: The data transferred from the detector are displayed here.
- 2) Data and file manipulations: File operations on datasets.
- 3) Evaluations of results: Graphic edition and manipulation with data.
- 4) Graphics on screen and printers: Creation and printing of graphs from datasets.
- 5) Notes: Display of calculated results, text edition and writing notes.
 - a) Technical notes: Some technical details of connecting detector to computer.
 - b) Error messages: Comments on error messages during execution of the program.
- 6) In addition to these common parameters a valuable kinetics program is also available within PZTools.

2.2.2.2 Stop flow measurements.

Two dense PAN crystals were labeled either specific or non-specific antibodies as described previously. Crystals were then placed into the flow cell and urine containing 15 ng/mL ricin was added by the peristaltic pump running at 1 mL/min for 20 seconds. The flow was stopped and the frequency changes were recorded under PZTools. As depicted in Figure no. 9, PAN coated QCM transducers detected 15 ng/mL of ricin in human urine against non-specific antibody coated transducers. When lower amounts of ricin were used, significance was lost between the signals.

2.2.3 Detection in saliva

2.2.3.1 Static measurements

Two dense PAN crystals, which were coated using the new masking process, were labeled with either specific or non-specific antibodies as described previously. Crystals were then placed into the flow cell and equilibrated with 50 μ L DPBS. Once frequencies were stabilized, 50 μ L of saliva containing 50 μ M PMSF and 20 ng/mL ricin was added and the frequency changes were recorded under PZTools. As depicted in Figure no. 10, PAN coated QCM transducers can detect this level of ricin in human saliva against non-specific antibody coated transducers. Dotted lines indicate a first order regression on both curves.

2.2.3.2 Stop flow measurements

Two dense PAN crystals were labeled with either specific or non-specific antibodies as described previously in section 2.2.1. Crystals were then placed into the flow cell and saliva as prepared in section 2.2.3.1 containing 5-20 ng/mL ricin was added by the pump as described in section 2.2.2.2 and the frequency changes were recorded under PZTools. Figure no 11 demonstrates detection of 20 ng/mL ricin in saliva. Figure 12 depicts a kinetics dose response curve generated by PZTools from 5, 10 and 20 ng/mL ricin in saliva

2.2.4 Detection in serum by static method

Two PAN crystals were labeled with specific or non-specific antibodies as described previously. Crystals were then blocked with either 1% BSA or 1% human serum for one hour at 37 °C. Crystals were washed as previously described. Crystals were then placed into the flow cell and undiluted or diluted serum containing 100 ng/mL ricin was added to the static side as described in section 2.2.2.1 and the frequency changes were recorded under PZTools. Figure 13 indicates detection with this method.

An unusual 3 Hz beat frequency was noted when performing these experiments. This could be due to the density of the undiluted serum. No specificity was noted with these experiments. (data not shown) Attempts to reduce the beat frequency as well as improve the assay were evaluated. The first objective was to identify any component which could be responsible for the unusual frequency response. The second objective is to remove those components from serum without

removing the biological ricin. To meet the first objective, we noted that serum contains very high amounts of high molecular weight protein, especially albumins. In addition to that, it also contains apolipoproteins which could also interfere with the assays. To reduce proteins the serum containing ricin was subjected to centrifugal spins with a 0.45 micron filtration tube. Filtrates were either saved or subjected to further filtrations. Aliquots of filtrate were then placed into a 30K molecular weight cut off vial and centrifuged further. Filtrate from these spins were also collected and stored for experimentation.

In spite of the extra filtration steps, the filtered spiked serum did not show any specificity for the biosensor. Beat frequencies were reduced, however this did not improve detections.

2.2.5 Detection in serum by drip and dry method

Crystals were treated as described in section 2.2.1. Five microliters of serum containing 100 ng or ricin was added to the crystal and the crystals were placed into a sealed temperature and humidity controlled incubator for one hour at 37°C. The crystals were washed in four 10 mL beakers of DPBS then four beakers of distilled water and dried. Frequencies were recorded for both the specific and non-specific crystals. Figure 14 demonstrates detection at this concentration. Due to time constraints, further experiments were not possible with lower ricin concentrations.

2.3 Protein G system

As described within the Phase I proposal, we were to co-evaluate other protein based matrices for the detection of ricin in urine and saliva. The use of protein G as a biological substrate is well established within this laboratory. As protein G has a high affinity for the goat and rabbit capture antibodies against ricin, it will make a good support matrix for this purpose. This data will be compared to PAN based detections to evaluate any significant improvement or differences between the two matrices in our detection formats.

2.3.1 Preparing the protein G crystals

Ten megahertz crystals were cleaned with 1.2M NaOH for 5 minutes, rinsed with distilled water and placed into 1.2M HCl for 5 minutes and washed as described. Dry crystals were then cleaned with concentrated HCl on the transducer portions only for 1 minute. The crystal were then washed with copious amounts of distilled water and dried. (It is thought that the oxidation of the gold transducer surface helps in the attachment of biological matrices such as protein A or G). Initial frequencies were recorded and 1 mg/mL solutions of protein G (recombinant) in DPBS were made. Five μ L protein G was applied to the top side of the gold transducer surface on the QCM crystal. Crystals were placed into a 37°C convection oven for 20 minutes, then placed into a fan forced 37°C oven for an additional 15 minutes. Dried crystals were removed, placed into desiccant-containing bags and stored overnight. The following day, crystals were washed in four 10 mL beakers of DPBS then distilled water and dried. Frequencies were recorded which represent the additional mass of the protein G on the transducer surface.

Crystals were then labeled with either 1mg/mL specific or non-specific capture antibodies against ricin. Specific crystals received 5 μ L of goat anti-ricin and non-specific crystals received 5 μ L goat anti-rabbit. Crystals were incubated for one hour at 37°C in a humidified atmosphere, then washed and dried as described previously and frequencies recorded.

2.3.2 Detections in saliva

2.3.2.1 Static measurements

Antibody labeled crystals were prepared as described in section 2.2.1 then placed into the flow cell and 50 μ L of DPBS is added and allowed to equilibrate. Fifty microliters of saliva, containing 20 ng/mL ricin and 50 μ M PMSF, was added and the frequency change was recorded under PZTools. Since significant frequency differences are noted within the first 500 seconds, longer time frames are not necessary for detection of this level of ricin in treated saliva. Noise depicted in the first few seconds of the specific and non-specific crystal is most likely due to the addition of saliva resulting in a density change of the hydrating solutions (DPBS). As the sample is not physically mixed, a short equilibration period most likely will result in noise of this type. This system did not yield significance from specific and non specific crystals. (data not shown)

2.3.2.2 Stop flow measurements

Two protein G crystals were labeled with either specific or non-specific antibodies as described previously in section 2.2.1. Crystals were then placed into the flow cell and saliva as prepared in section 2.3.2.1 containing 5, 10 or 15 ng/mL ricin was added by the pump, the flow was stopped and the frequency changes were recorded under PZTools. A resulting dose response curve is demonstrated in figure 15.

2.3.2.2.1 Kinetic analysis

In order to speed up the detection process and understand more closely the interaction of the toxin ricin with its corresponding antibody, we have performed kinetic analyses. Under PZTools software, we can compare the binding constants $k(as)$ of the toxin to the QCM. The first 100 seconds of data is selected under a datasets window from the known concentration of ricin. Association curves are generated from the dose response and plotted as the change in slope (Hz/sec) versus the toxin concentration. Graphics created under this software can be exported as a metafile to other programs for further analysis.

In Figure no.12, three concentrations of ricin (ng/mL) are depicted against slopes from their respective binding curves. Large standard errors are the result of one binding curve per ricin concentration being used for calculation. This is the minimum data necessary for generation of kinetic constants and improves when additional datasets are added for calculations.

2.3.3 Detection in serum

2.3.3.1 Static measurements

Crystals were labeled with antibody as described in section 2.3.1. Detections were as described under section 2.2.2.1 with the inclusion of serum rather than urine. Some of the serum used in this section was treated differently than described earlier in this report. (This will be described later in this section) Crystals were then blocked with either 1% BSA or 1% human serum for one hour at 37 °C. Crystals were washed as previously described. Crystals were then placed into the flow cell and undiluted or serum diluted 1:2 diluted 100 ng/mL ricin was added by the pump, the flow was stopped and the frequency changes were recorded under PZTools.

An unusual 3 Hz beat frequency was noted when performing these experiments. This could be due to the density of the undiluted serum. No specificity was noted with these experiments. Attempts to reduce the beat frequency as well as improve the assay were evaluated. The first objective was to identify any component which could be responsible for the unusual frequency response. The second objective is to remove those components from serum without removing the biological ricin. To meet the first objective, we noted that serum contains very high amounts of high molecular weight protein, especially albumins. In addition to that, it also contains apolipoproteins which could also interfere with the assays. To reduce proteins the serum containing ricin was subjected to centrifugal spins with a 0.45 micron filtration tube. Filtrates were either saved or subjected to further filtrations. Aliquots of filtrate were then placed into a 30K molecular weight cut off vial and centrifuged further. Filtrate from these spins were also collected and stored for experimentation.

In spite of the extra filtration steps, the filtered spiked serum did not show any specificity for the biosensor. (Data not shown) Beat frequencies were reduced, however, this did not improve detections.

2.3.3.2 Stop flow measurements

Crystals were labeled with antibody as described in section 2.3.1. Detections were as described under section 2.2.2.2 with the inclusion of serum rather than urine. Serum used in this section was treated differently than described earlier in this report. Undiluted human serum was spiked with known amounts of ricin then subjected to exclusion membrane centrifugation. Specifically, spiked serum was added to a 0.45 μ M filter to remove particulate. The microtube was spun at 5000 RPM until filtered and the filtrate was used for experimentation. All other experimental procedures were as described in section 2.2.2.2. In spite of the extra treatments of serum, this method did not produce detection. (Data not shown)

2.3.3.3 Drip and dry measurements

Crystals were prepared as described in section 2.3.1. Experimental procedures were as described in section 2.2.5. Figure 16 depicts detection of 100 ng/mL ricin in serum.

2.3.4 Detection in urine

2.3.4.1 Static measurements

Not performed

2.3.4.2 Stop flow measurements

Two protein G crystals were labeled either specific or non-specific antibodies as described previously in section 2.3.1. Crystals were then placed into the flow cell and urine containing 20 ng/mL ricin was added by the pump as described in section 2.2.2.2, the flow was stopped and the frequency changes were recorded under PZTools. As demonstrated in figure no. 17, 20 ng/mL ricin in urine is detectable with this method.

3.0 Conclusions.

3.1 General considerations

As demonstrated by the results presented within this final report, the detection of ricin in biological matrices is possible utilizing both ELISA and PAN or protein G-based piezoelectric biosensors. The biosensors are relatively specific and do not require additional reagents for signal generation. In addition, the results can be identified within seconds of the tests start. Ease of use is one of the most important parameters when reviewing a new method or process in detection of biologicals. The piezoelectric detection methods described herein demonstrate that simple addition of untreated or minimally pretreated samples of biological matrices can result in identification of toxins such as ricin.

These types of assays are important to clinicians as well as other less highly trained ARMY personnel. They provide a relatively simple assay that can be performed without expensive instrumentation or highly trained personnel. These biosensor assays also have the advantage of being deployed in-field. This could be important when reference laboratories are located in remote areas away from the testing site making their use unfavorable. It could be envisioned that assays of this type could be used in combat or in areas where no other means of biological warfare identification are available.

Secondary to the development of the ricin assay, we have also demonstrated that biological matrices, such as serum, saliva or urine, can be used in the detection format. We expect that other analytes such as biological warfare agents, bacteria or viruses can be detected in serum, saliva or urine with the piezoelectric methods described in this report. As the specificity of the sensor is directed by the capture antibody, one only needs to switch the labeled crystal to redirect the assay for a new analyte. We have demonstrated detection in human biological matrices which reflects likely reactions when other analytes are chosen for detection. No significant fouling of the sensor nor non-specific reactions occurred during the developmental period. This demonstrates the utilitarian benefits which should transfer to other assays if conceived.

Other methods exist or are in development for piezoelectric detection of biologicals. The Principal Investigator of this project has recently described a competitive assay for the detection of *Listeria monocytogenes* in food matrices which can be run in as little as five minutes.¹⁸ He has also described piezoelectric detection of toxin producing or pathogenic bacteria, such as *Vibrio cholerae* O139, based on the capture assay procedures described within this report.¹⁹ The wide range of possible analytes, together with assay speed and simplicity, demonstrate the versatile nature of piezoelectric biosensors which warrant further investigation.

3.2 Problem points

The assay of ricin in serum has not been successful due to the unusual beat frequencies when recording data. It is unknown how this occurs, but it may be due to the density or viscosity of the media or to other factors when using serum. IgG levels in serum are 10X higher than what is immobilized on the transducer. If the protein G is not saturated, the extra serum immunoglobulins could react and cause a non specific attachment of the serum analytes to the transducer. This non specific attachment of IgG may inhibit the other antibodies directed against the analyte from reactions.

Remedies for this beat frequency may include the following. 1) Separation of the serum IgG from the matrix using molecular weight exclusion membranes. Short centrifugal spins using molecular weight cutoffs which allow ricin to pass through separate serum IgG. Protein G columns^h could also be used to remove serum IgG from the matrix. Unigue products are now on the market which would allow for serum to be passed through, binding the immunoglobulins, and leaving other serum components intact. Dilution of the serum is not considered to be a viable alternative as this would also dilute toxins found within that matrix.

3.3 PAN crystal preparation problems

Machinery that was used to prepare PAN crystals broke down in the last month of this project. Repair of the instrument was not possible in time to supply PAN crystals for the last section of research with serum. Serum detections were carried out on protein G coated crystals to demonstrate that detections such as these are possible with our detection system.

3.4 Dual use possibilities

Likely areas where these types of sensors could be used in a dual use setting include: 1)Clinical laboratories where rapid methods of detection are necesasary. 2)In-field detections where clinical laboratory support is limited or non-existant. 3)Food manufacturing or processors, where rapid detection of food spoilage organisms or the toxins that they produce improves freshness and safety of consumables. 4)Environmental monitoring where in-field detection could identify pollutants and or their sources.

^h Protein G acti-disk 25. Whatman, Inc. Clifton, NJ.

5.0 References:

- 1 Ember, L. Chemical and Engineering News, p.9-10. April 22, 1991.
- 2 Roederer, J.E. and Baastians, G.J. Anal Chem. 55, 2333. 1983.
- 3 Plomer, M., Guilbault, G.G. and Hock, B. Enzyme Microb. Technol. 14. 230. 1992.
- 4 Prusack-Sochaczewski, E., Luong, J.H., and Guilbault, G.G. Enzyme Microb. Technol. 12, 173. 1990.
- 5 Jacobs, M.B., Carter, R.M., Lubrano, G.J. and Guilbault, G.G. 1995. American Laboratory 27(11). 26. 1995.
- 6 Konig, B. and Gratzel, M. Anal Chem. 66 (3). 341. 1994.
- 7 Ebersole, R.C., Miller, J.A., Moran, J.R. and Ward, M.D. J. Amer Chem Soc. 112. 3239. 1990.
- 8 Yamaguchi, S., Shimomura, T., Tatsuma, T., and Oyama, N. Anal Chem. 65. 1925. 1993.
- 9 Minnuni. M., Mascini, M., Guilbault, G.G. and Hock, B. Anal Lett. 28 (5). 749. 1995.
- 10 Henry, C. Anal Chem. 68. (19). 1996. (In press)
- 11 Lundquist, N-H. FOA Orieterar OM. Liber Distribution. Stockholm, Sweden. 1983.
- 12 Hewetson, J. F., et al. Vaccine. 11. 743. 1993
- 13 Carter. R.M., Lubrano, G.J., and Guilbault, G.G. Life Chemistry Reports. 11. 271. 1994.
- 14 Poli, M.A., Rivera, V.R., Hewetson J.F. and Merrill, G.A. Toxicol. 32(11). 1371. 1994.
- 15 Carter, R.M., Jacobs, M.B., Lubrano, G.J., and Guilbault, G.G. Anal Lett. 28(8). 1379. 1995.
- 16 Sauerbrey, G.Z.Z. Physik. 155. 206. 1959.
- 17 Koblinger, E. et al. Sensors and Actuators B 24-25. 107. 1995.
- 18 Minnuni, M., Mascini, M., Carter, R.M., Jacobs, M.B., Lubrano, G.J., and Guilbault, G.G. Anal Chim Acta. 325. 169. 1996.
- 19 Carter, R.M., Mekalanos, J.J., Jacobs, M.B., Lubrano, G.J., and Guilbault, G.G. J. Immunol Meth. 187. 121. 1995.

Ricin Capture ELISA (Ab comparison)

(10 min color)

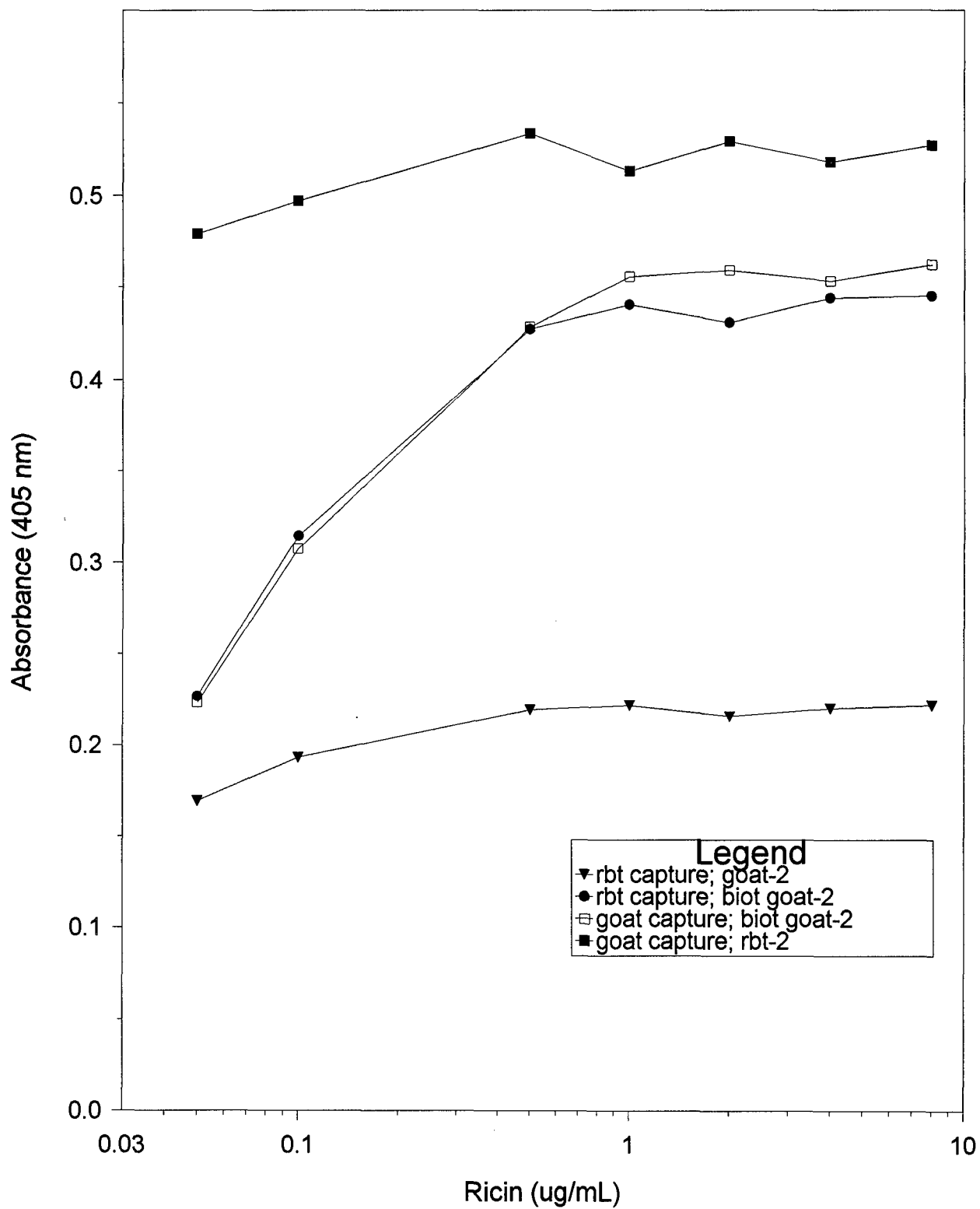


Figure 1

Ricin Capture ELISA (Ab comparison) in Saline

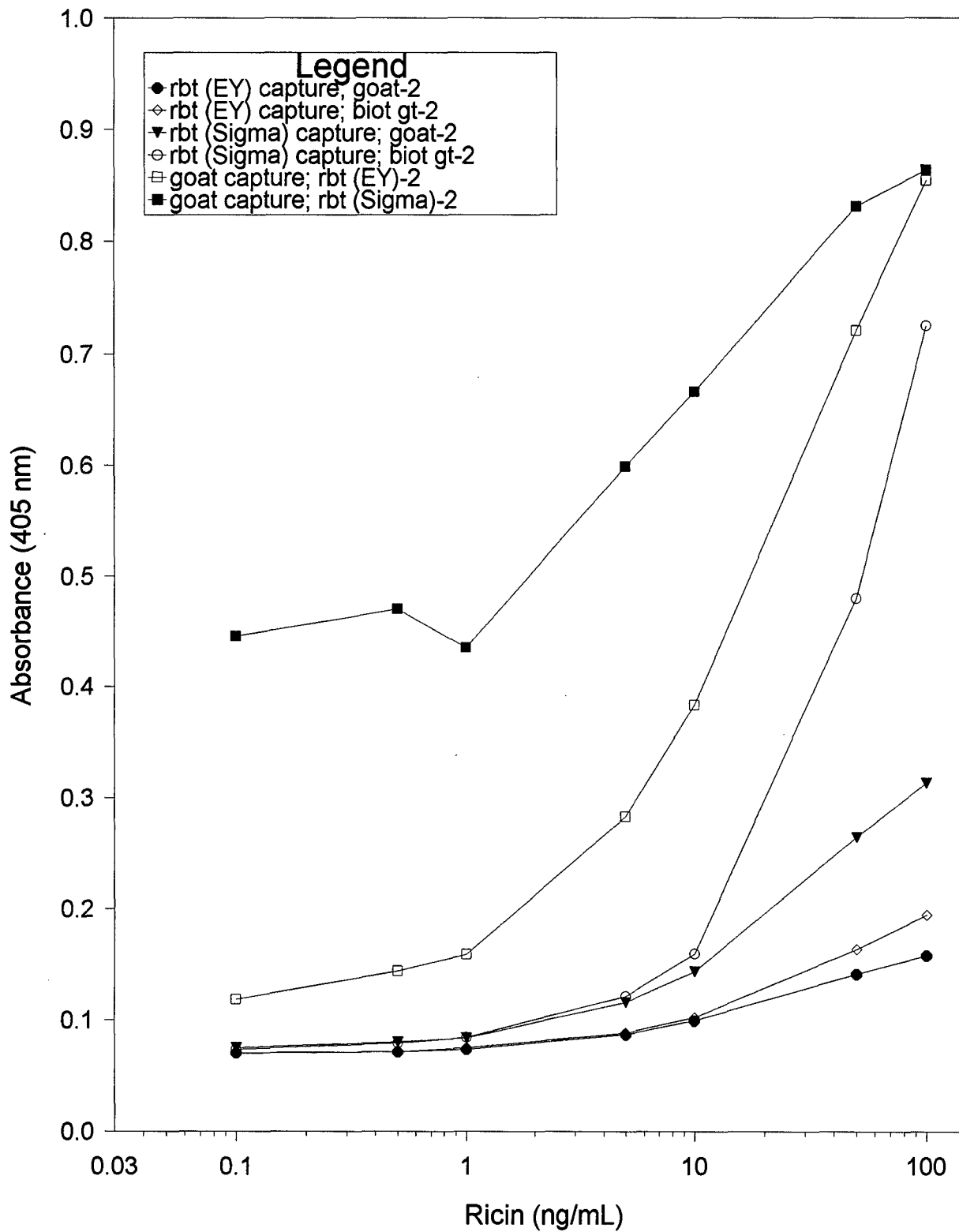


Figure 2

Ricin Capture ELISA in Urine (Ab comparison)

(20 min color)

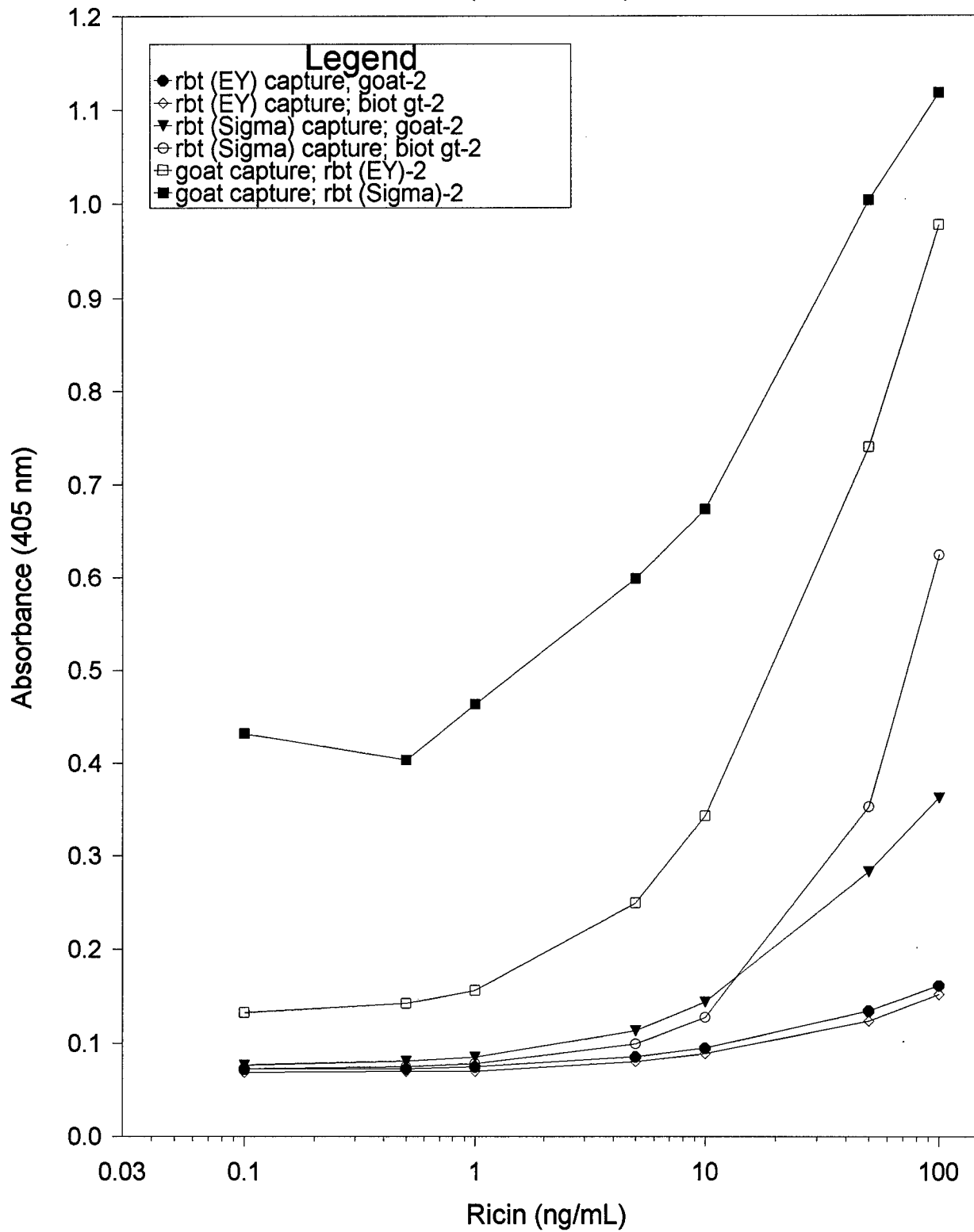


Figure 3

Ricin ELISA (Sigma Rabbit Capture)

(Ricin in DBT vs. Urine)

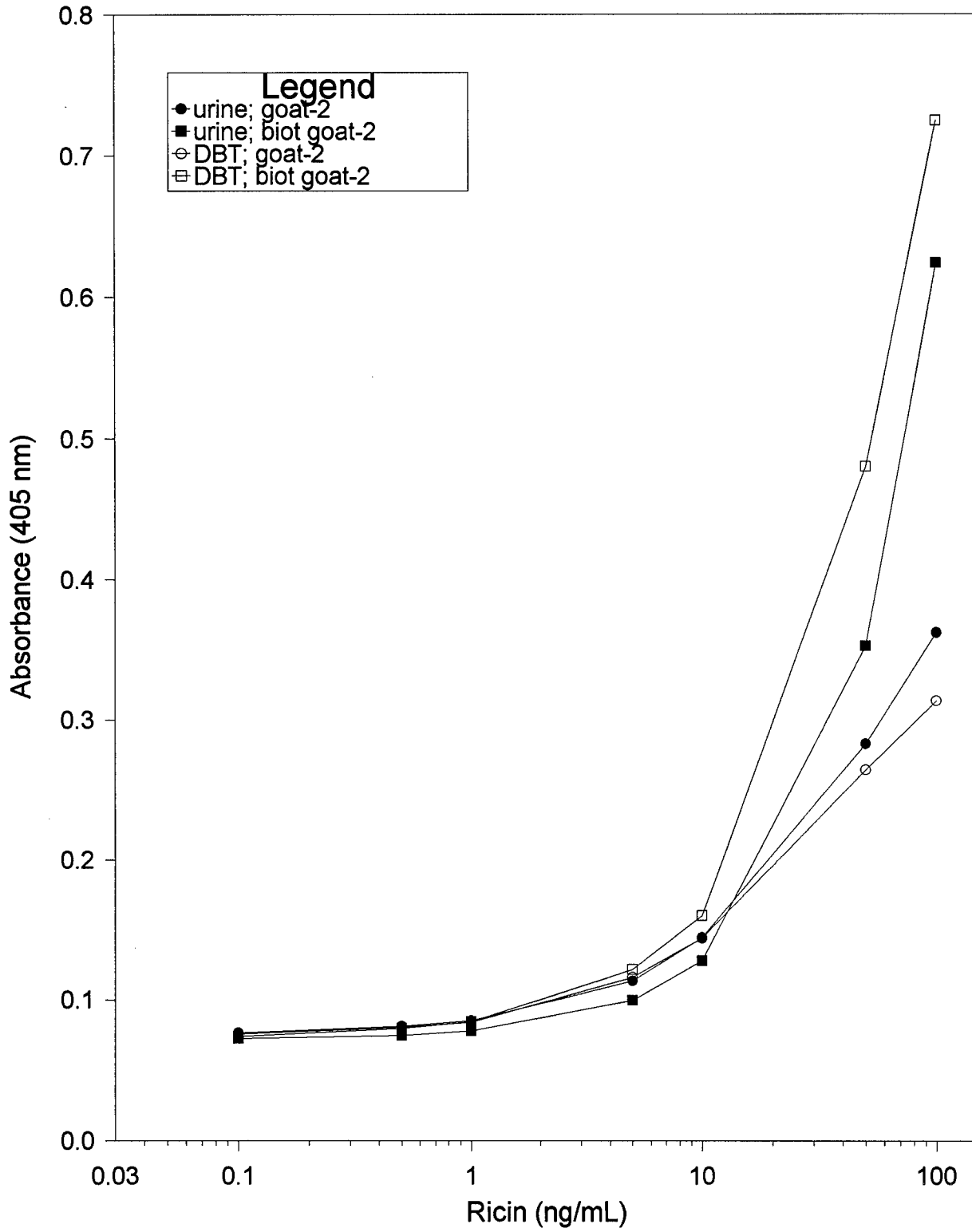


Figure 4

Ricin ELISA (EY Rabbit Capture)

(Ricin in DBT vs. Urine)

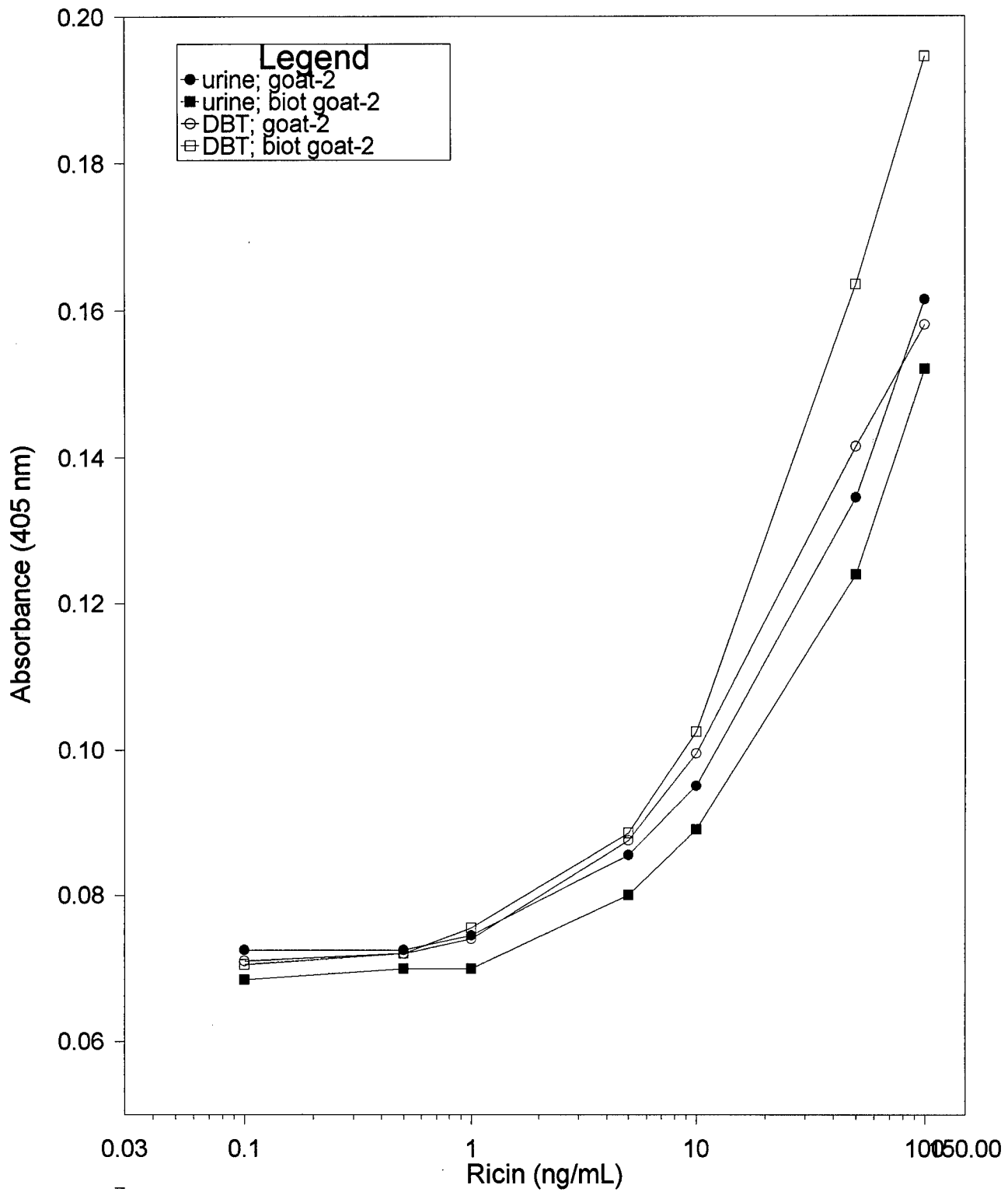


Figure 5

Ricin ELISA in Saliva

(Goat capture; Sigma rabbit-secondary Ab)

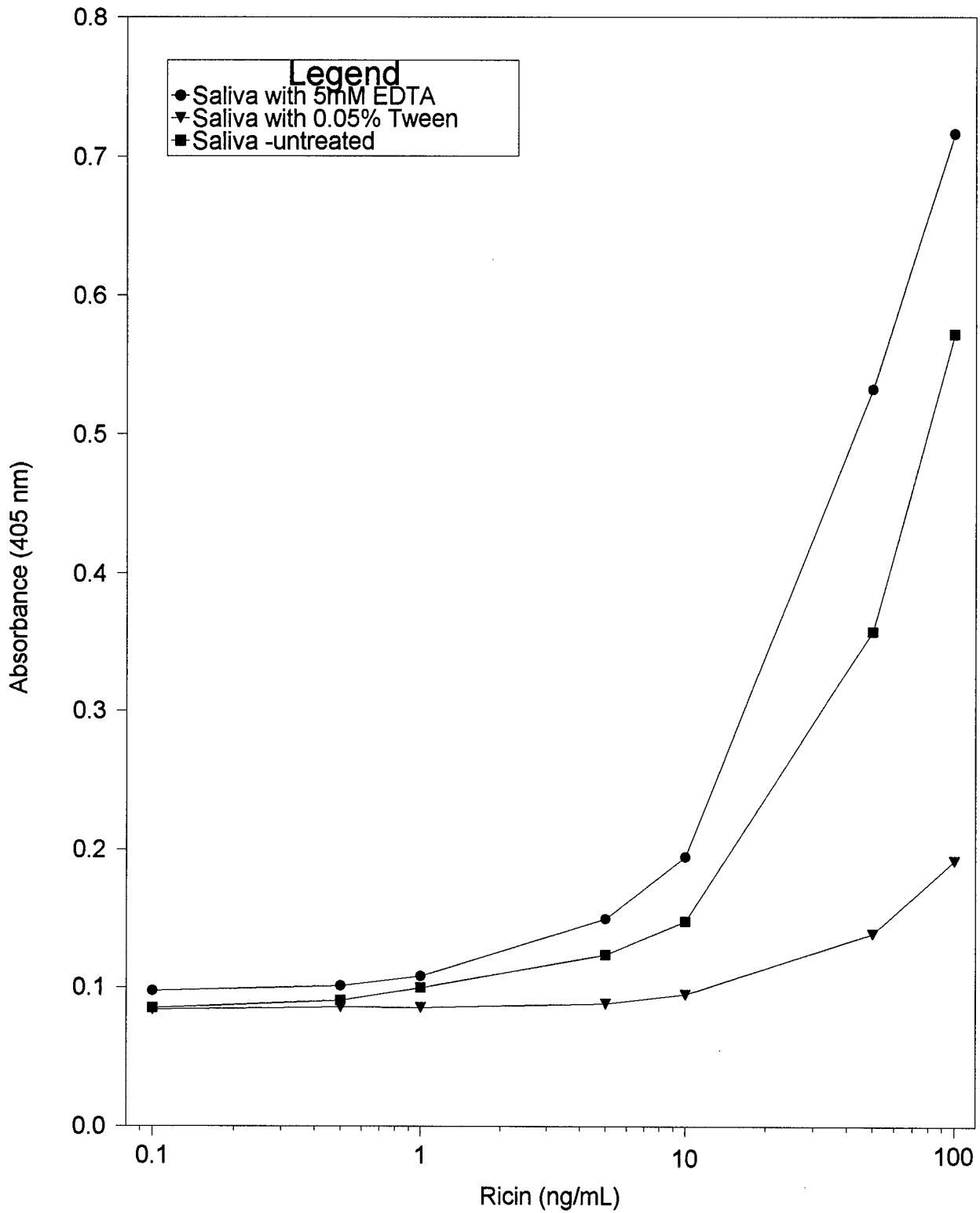


Figure 6

Ricin ELISA in Saliva

(goat capture; Sigma rbt- 2nd Ab)

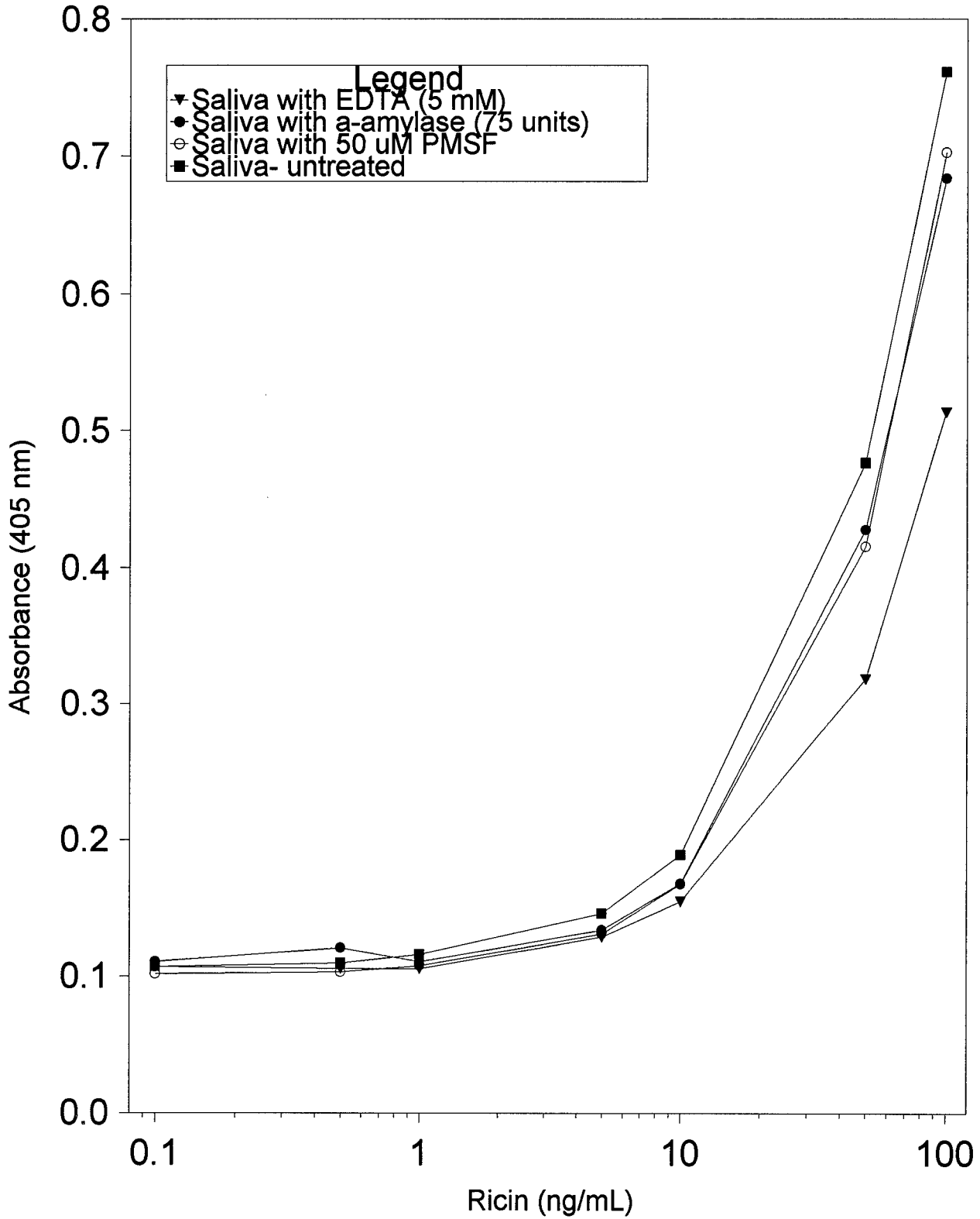


Figure 7

Ricin ELISA in Human Serum

(Goat capture; Sigma rabbit-secondary Ab)

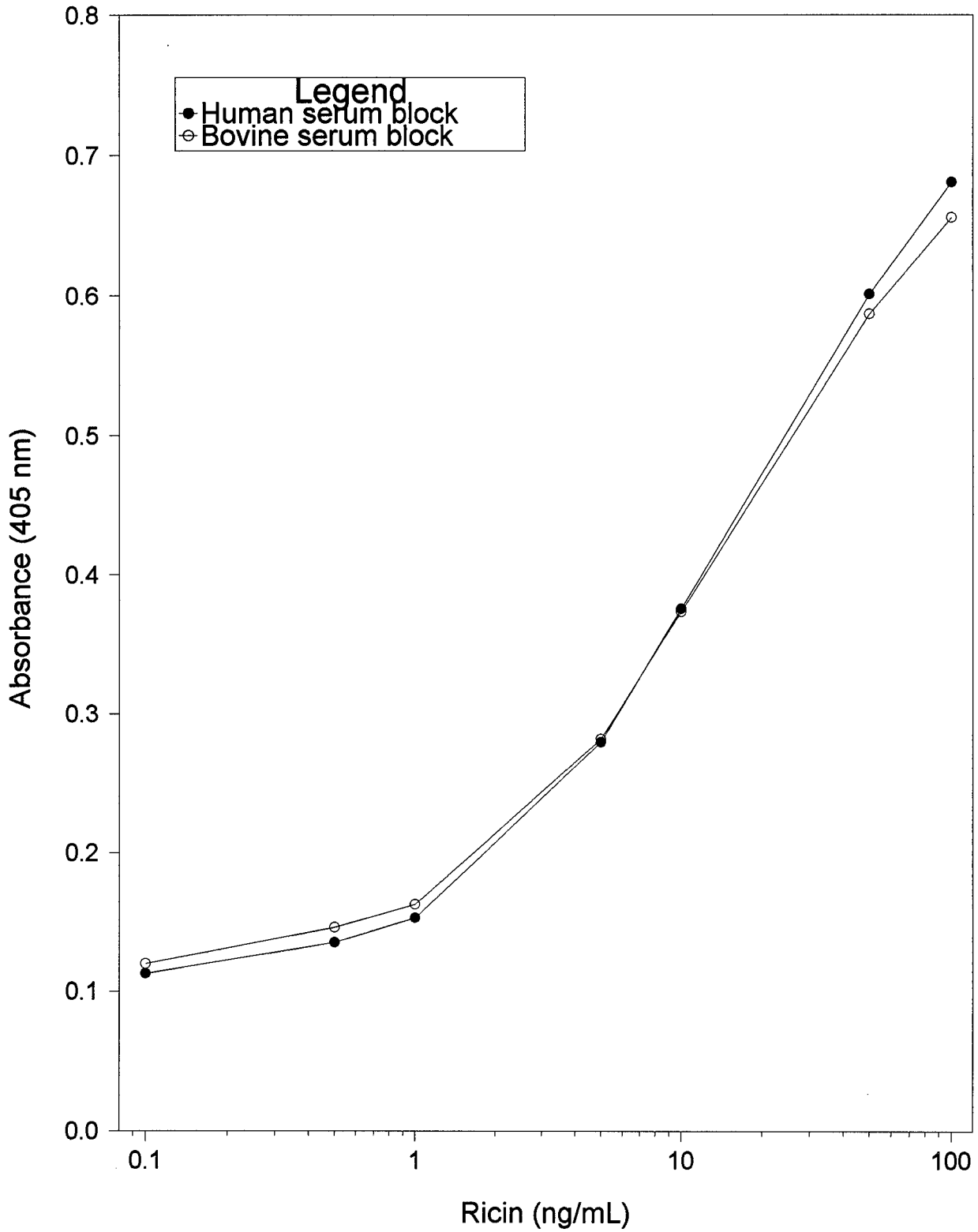


Figure 8

PAN QCM Detection of 15 ng/mL Ricin in Urine

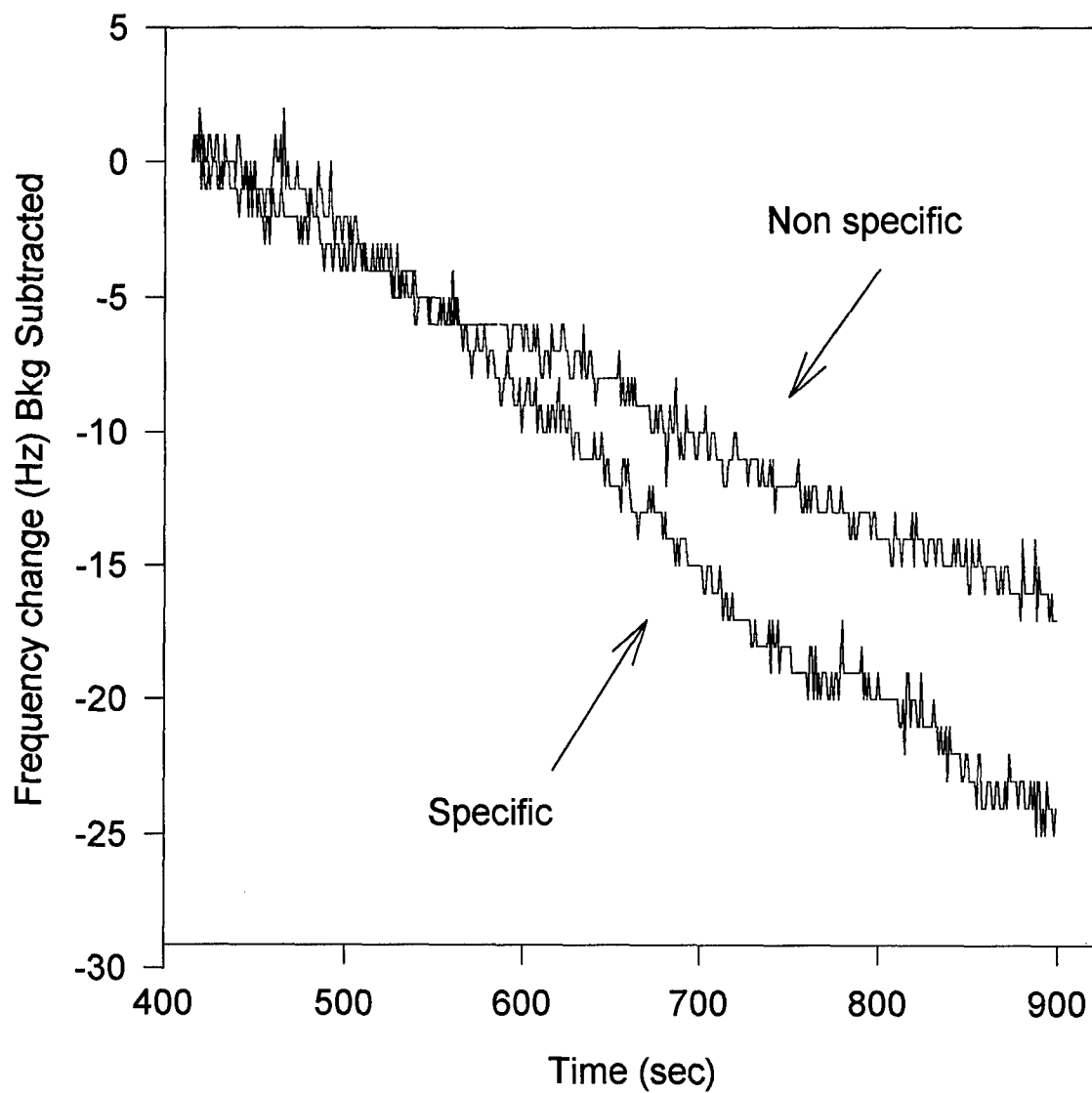


Figure 9

PAN QCM Detection of 20 ng / mL Ricin in Saliva

Saliva treated with 50 μ M PMSF

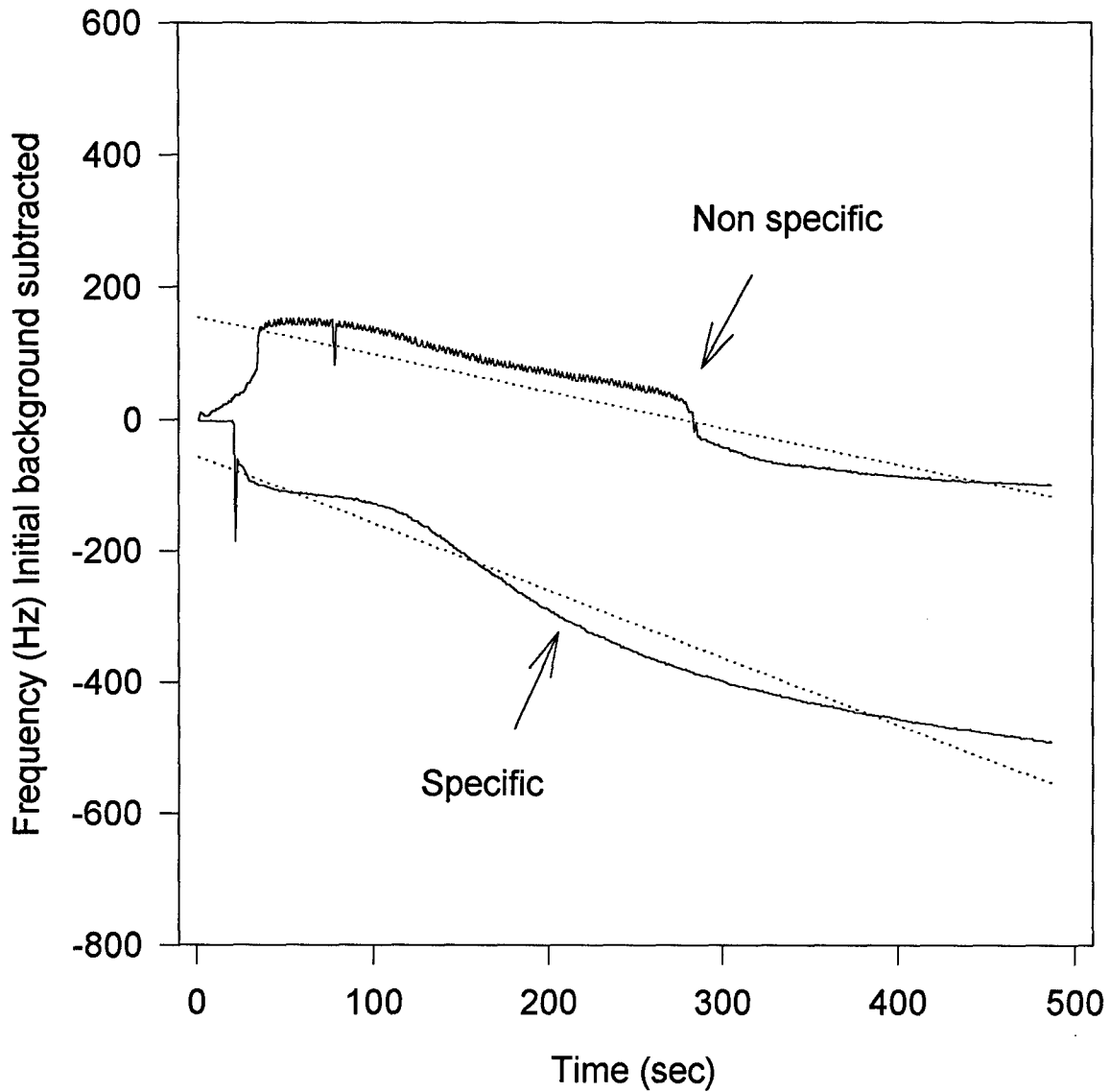


Figure 10

PAN QCM Detection of 10 ng/mL Ricin in Saliva
Stop flow method

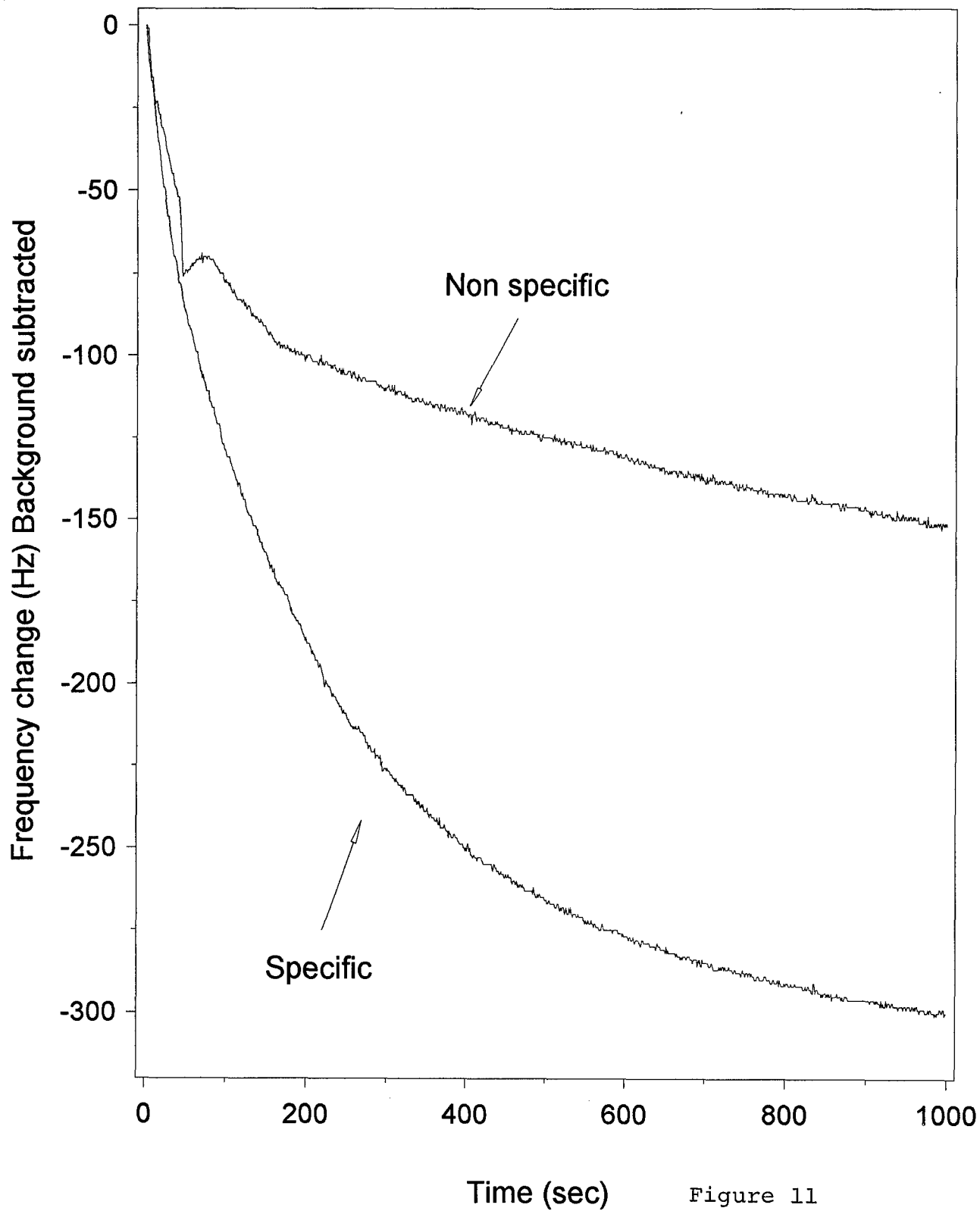
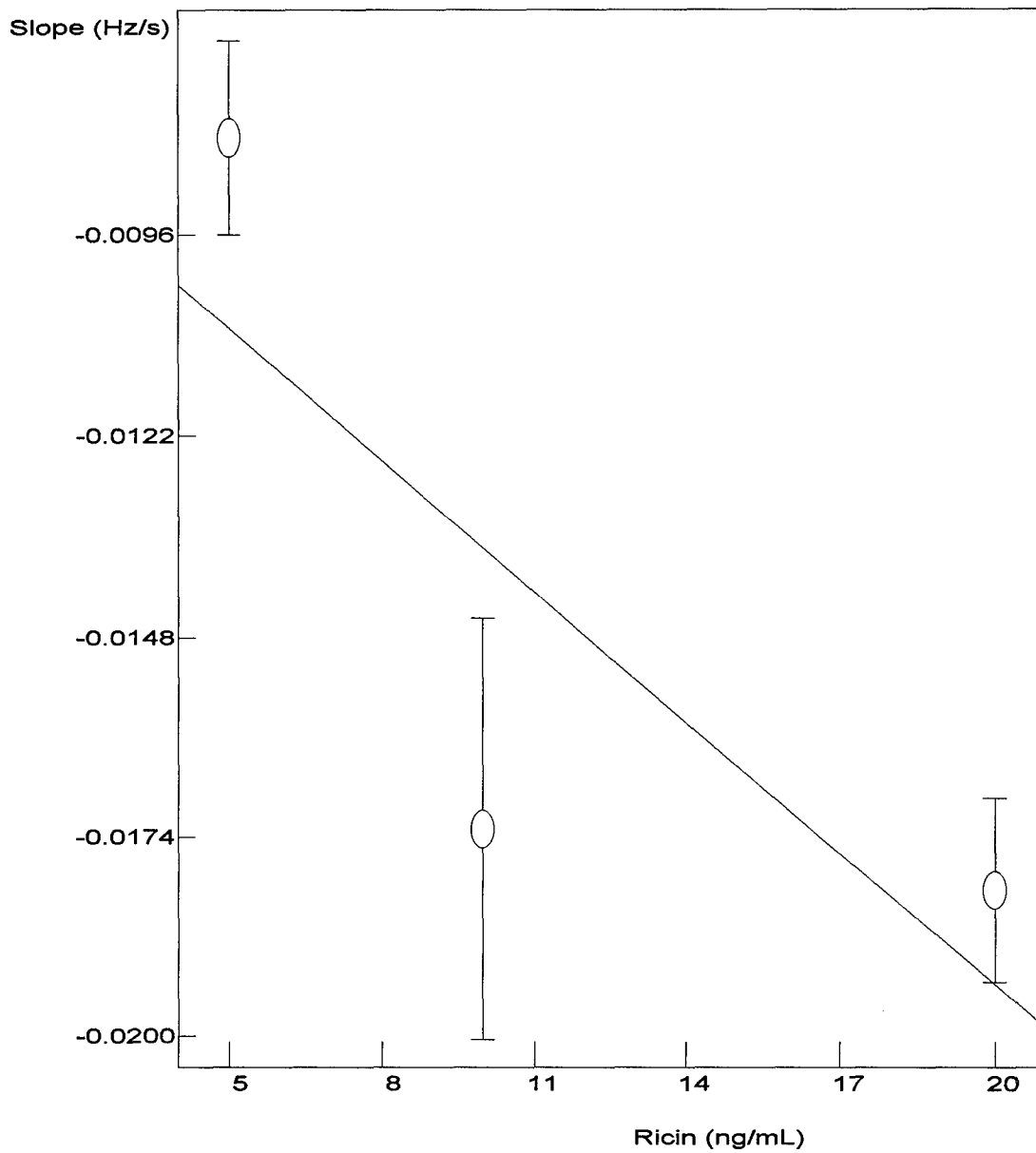


Figure 11



Kinetic rate constants

k(as): 0.00056726 (74.5%) XU-1 s-1

k(dis): 0.00796854 (70.2%) s-1

f(max): -104.8 Hz

Figure 12

Static Detection of 100 ng/mL Ricin

50/50 Serum-DPBS

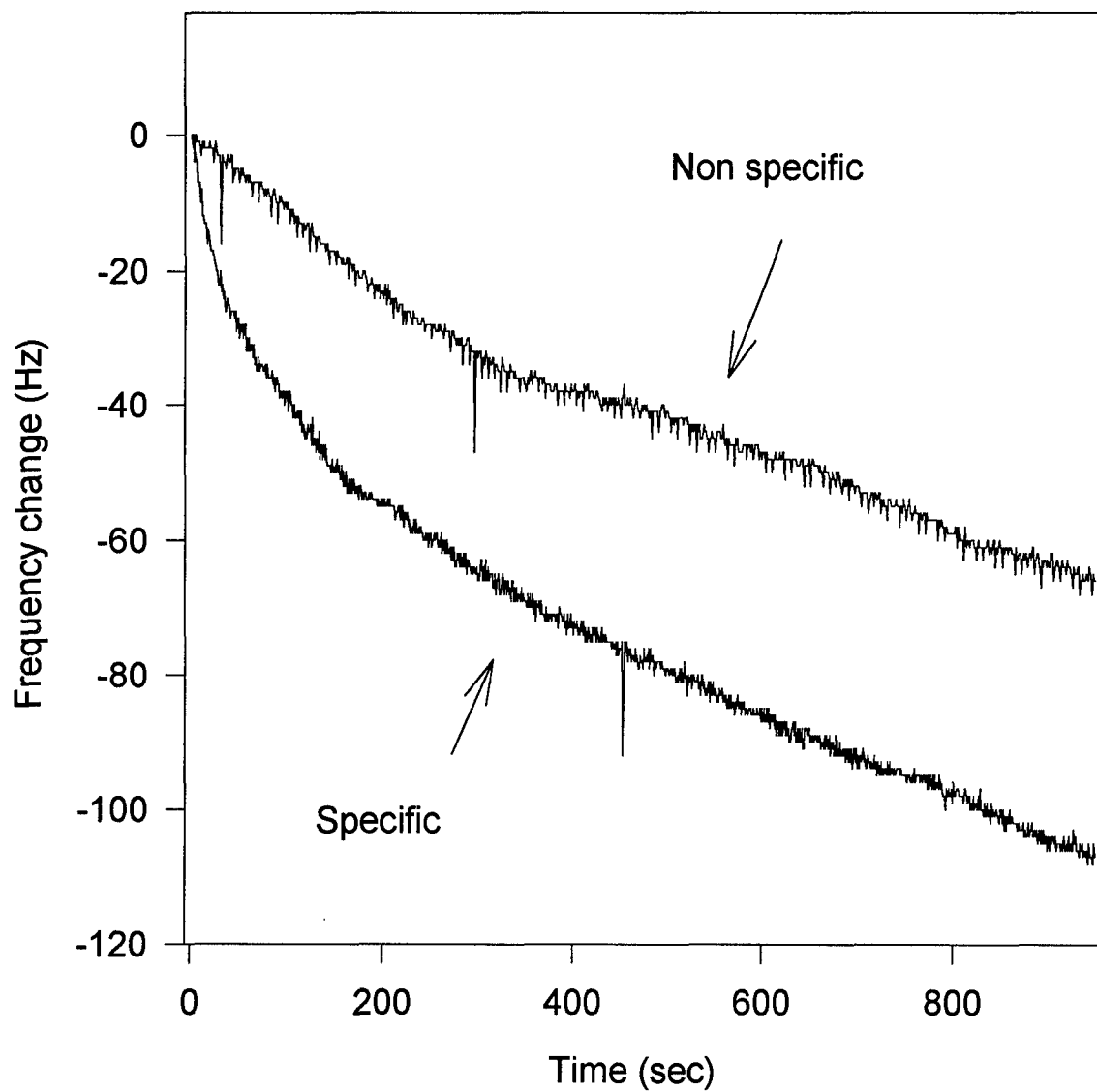


Figure 13

PAN/QCM Detection of Ricin in Human Serum

(100 ng/ml ricin in 50/50 spun HS/DPBS)

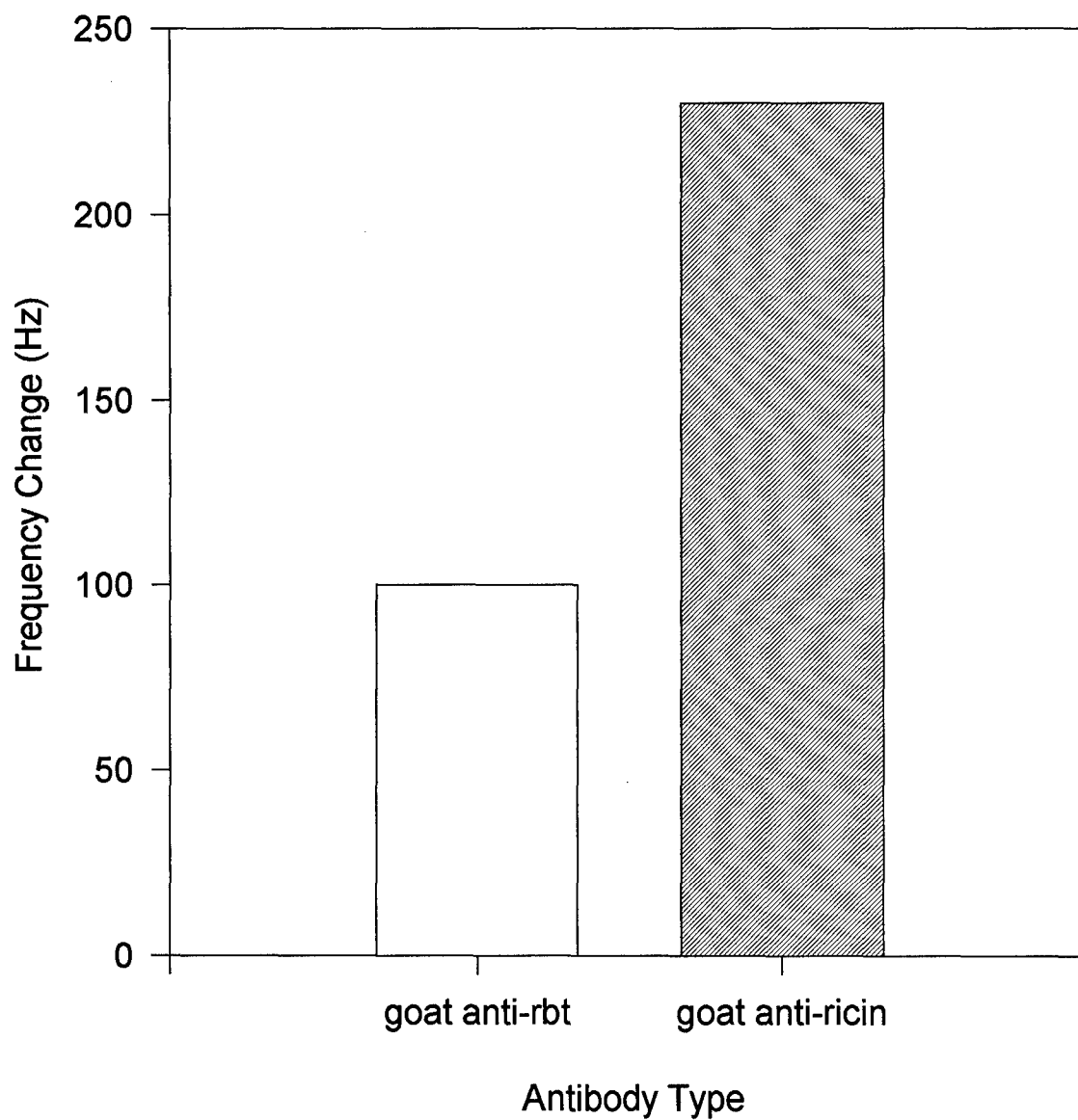


Figure 14

Piezoelectric-based Ricin Detection (Dose Response)
In Human Saliva

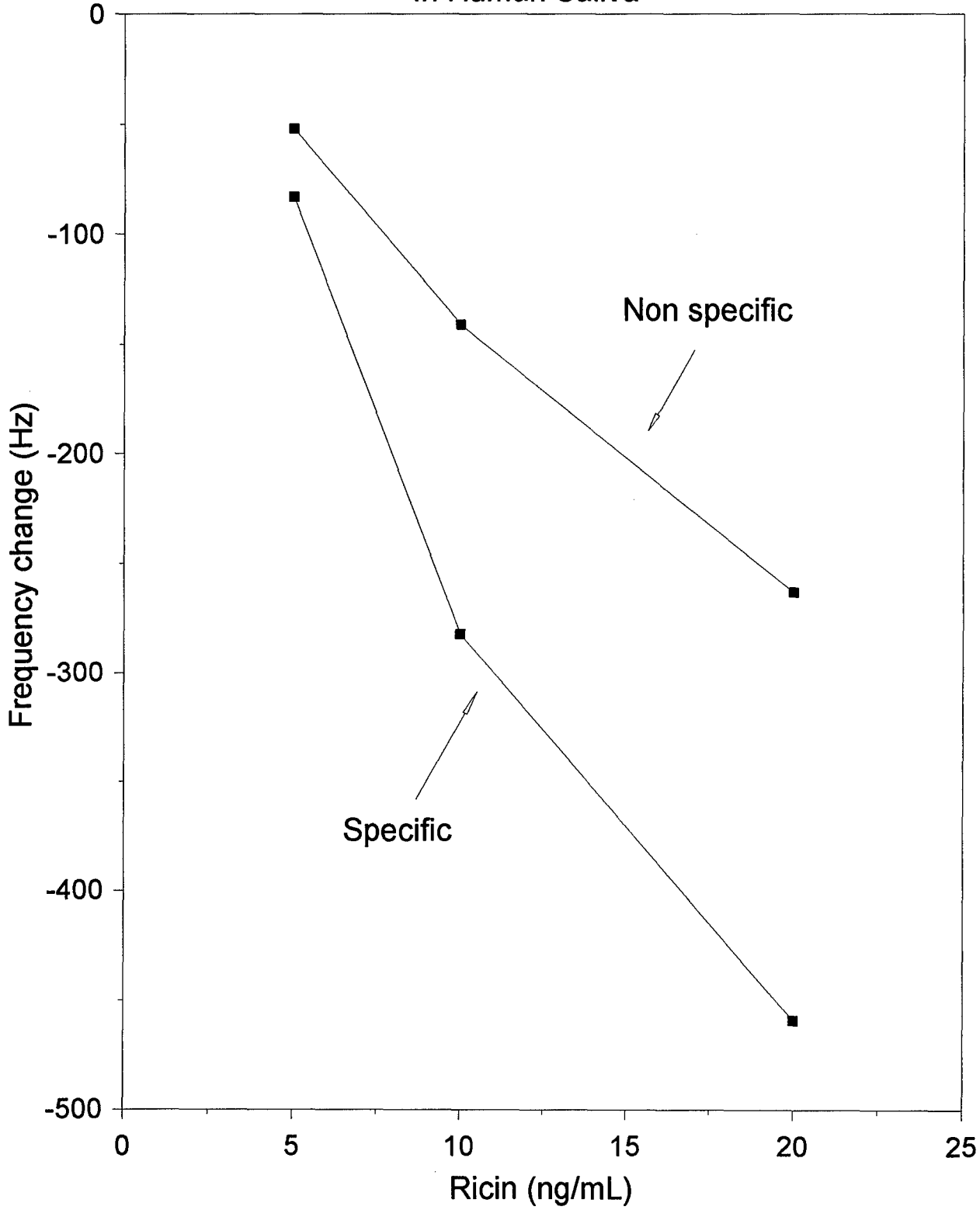


Figure 15

PZ Detection of Ricin in Human Serum

(100 ng/ml ricin in 50/50 spun HS/DPBS)

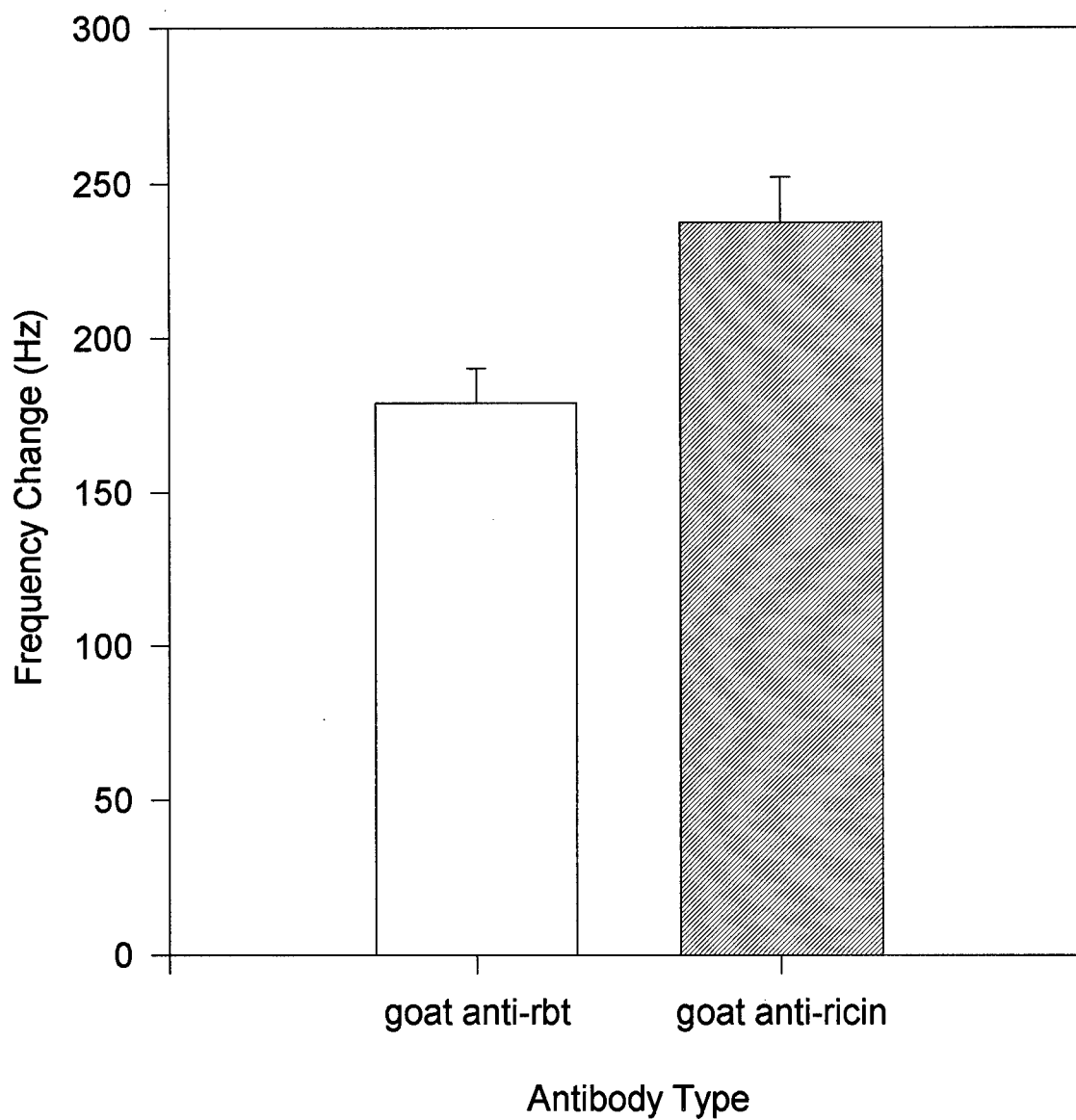


Figure 16

Piezoelectric Detection of 20 ng/mL Ricin In Human Urine

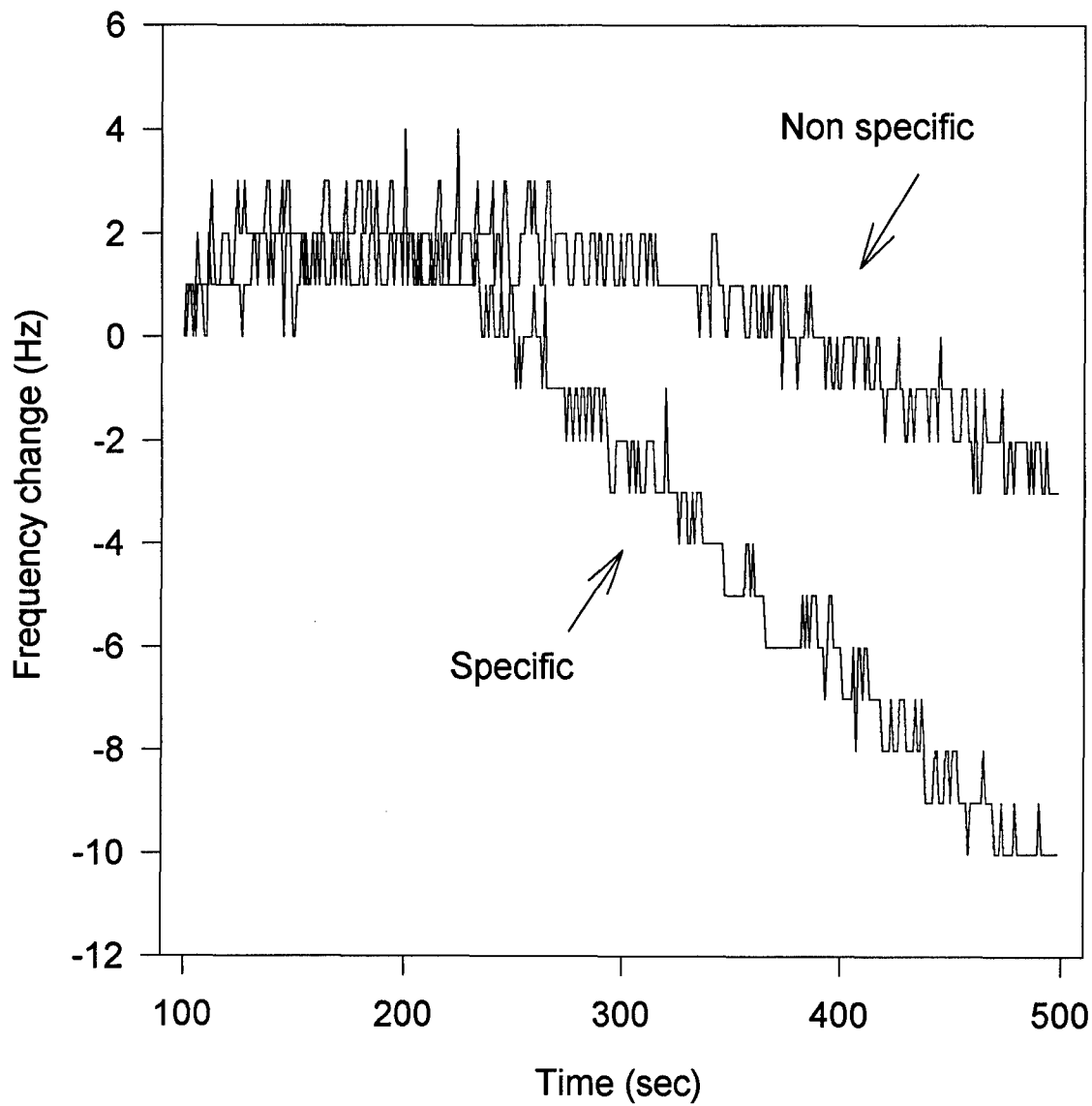


Figure 17



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ADB227451	
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