Part 70-03

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FINAL REPORT

A FEASIBILITY STUDY FOR THE DEVELOPMENT OF A BIOLUMINESCENT NARCOTICS DETECTOR

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PREFACE

Under the joint sponsorship of the New York Police Department, New York, and the Law Enforcement Assistance Administration, Washington, D.C., RPC Corporation conducted a six-month feasibility program utilizing a microbial sensor to achieve the rapid and specific detection of heroin and marijuana. A companion program was conducted utilizing additional microbial sensors to achieve the rapid detection of explosives.

RPC Corporation wishes to acknowledge the assistance provided by the Narcotics Division, New York Police Department, and the United States Army Land Warfare Laboratory, Aberdeen, Maryland.

SUMMARY

The use of microbial sensors has been demonstrated as a reliable, specific, and rapid method of detecting low concentrations of a variety of gases in several previous and current programs sponsored by The U.S. Government. Thc concept of this detection system is based on the ability of bioluminescent microbial sensors to react to various gases.

Studies were conducted during a six month program to prove the feasibility of using microbial sensors for the rapid and specific detection of heroin and marijuana vapors. The program consisted of the following: a survey and analysis of the background environment; selection and isolation of suitable microbial biosensors; analysis of various growth and response parameters of these sensors; development of biosensors with increased sensitivity; and several demonstrations of feasibility. Additionally, laboratory instrumentation was designed and fabricated to aid in the conduction of the various phases of the program. The results of these studies are the subject of this report. For convenience, a glossary of technical terms and word usage is included.

A study of the background environment was made during an 11 (eleven) day period in New York City; various sites throughout the city, all with a history of narcotics problems, were surveyed. No locations were found with a normal gaseous environment which might interfere with the detection of narcotics.

Microbial biosensors from various sources including the RPC Corporation Culture Collection, the American Type Culture Collection, and various domestic and foreign natural sources were selected and isolated for this program. Strains showing sensitivity to narcotics were retained for

further studies, while those strains without demonstrable sensitivity were not studied further.

Studies of various growth and response parameters were made on those strains initially showing sensitivity to heroin and/or marijuana. The studies included analysis of the effects of age, growth medium formulations, intensity, sampling flow rate, temperature and relative humidity, and drug dependence. Collectively, the results were utilized to select those strains with superior operational characteristics.

Additionally, a strain selection task was conducted utilizing those strains selected on the basis of preliminary tests. This task, utilizing various mutagenic and physiological state-of-the art techniques, was designed to increase the sensitivity and/or improve other operational characteristics (useful life, selectivity, etc.) of strains previously selected. Additional analyses of the strain's response to various narcotic simulants, cutting agents, and controls were performed to ascertain discrimination capabilities of the individual strains.

The combination of the above techniques and studies resulted in the selection of two superior strains of biosensors for use in the feasibility demonstrations. These strains consistently exhibit excellent, repeatable detections and discrimination between heroin and the controls and were used in several demonstrations, both in the RPC Corporation laboratories, the New York Police Academy, and New York City Hall. Although several strains have shown some degree of sensitivity, none exhibiting repeatable sensitivity to marijuana were isolated during the course of this program.

Laboratory instrumentation fabricated for this program consisted of a test apparatus designed to allow the simultaneous evaluation of up to six biosensors. The apparatus, completely self-contained with the exception of a recorder, was used for the majority of the laboratory studies in this program. The results obtained with this apparatus were reproducible and correlated well with other RPC Corporation detector systems, providing further evidence of the specific sensitivity of the selected biosensor strains.

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BACKGROUND INFORMATION

Bioluminescence and Biosensors

Bioluminescence, the emission of visible electromagnetic radiation or light by living organisms, is a naturally occuring phenomenon among diverse species of organisms. Microbial species capable of this light emission include bacteria, protozoa, and fungi. Historically, the first study of microbial luminescence was in the late 19th century. Since that time it has been the subject of diverse applied and academic research programs. The occurence of this light emission has been shown to be a normal metabolic process, and the biochemistry of the process is known in some detail. It has been shown, for bacterial and fungal forms, to be dependent on the organisms' gaseous environment.

A characteristic of life, by definition, is the ability to respond to changes in the environment. The concept of a biosensor is based on this characteristic, and a biosensor may be defined as a living organism whose characteristic response to a specific change in the environment is indicative of the change itself. Microbial luminescence, being dependent on the gaseous environment, is thus useful as a sensor for changes in the gaseous environment. The luminous organisms may be considered a vapor sensor or chemical-optical transducer.

Detection Systems

Use of a photosensor or optical-electrical transducer, in conjunction with a gas sampling system and the biosensor, forms an elementary type of detection system. The system depends on monitoring the light output of the

bioluminescent culture, as samples of gas are passed across its surface. This monitoring results in the generation of an electrical signal carrying chemical information. Under ideal conditions, the signal varies proportionately with the specific environmental change.

Originally developed in 1962 by Dr. A. Thanos to monitor toxic gas levels in the Apollo Command Module Atmosphere, the bioluminescent detector concept has been applied, by RPC Corporation, to such diverse detection requirements as pesticides, jet fuels, explosives, and most recently narcotics. These diverse capabilities are possible because individual strains of biosensors respond, in a reproducible manner, to certain chemicals while others do not respond, or respond differently to different chemicals. Furthermore, certain biosensors are capable of responding to classes of chemicals in general, while still differentiating between the members of the class.

The outstanding features of utilizing bioluminescence for vapor detection are its sensitivity and rapid response. The sensor element is good for multiple exposures, has a useful life measurable in hours to days, and is easily replaceable. Additionally, the detection system contains very few moving parts, primarily those concerned with air movement for both sampling and environmental control of the biosensor. State-of-the art technology at RPC Corporation permits real-time detection of lower than parts per million concentrations of various gases, utilizing both portable and stationary detectors.

ENGINEERING DEVFLOPMENT

The design and fabrication of a multi-sensor laboratory test apparatus was accomplished as the major contractual engineering task. This apparatus was designed to allow the rapid and repeatable testing of biosensors as required in the various tasks of the program. The laboratory test apparatus, shown pictorially in Figures 1 and 2, and schematically in Figures 3 and 4, is basically an integrated test system which allows the simultaneous analysis of up to 6 (six) biosensors to a given effluent. The apparatus consists of several subassemblies: the sampling network, the photochemical sensor units (PSU), and the various electronic circuitry. Details of the design philosophy and operation of the apparatus and its component parts, are discussed below.

Gas Sampling System

The component parts of the gas sampling system of the laboratory test apparatus are shown schematically in Figure 3. Sampling is effected by insertion of a stainless steel probe into the test effluent container for the desired time interval. The sampled effluent flows through a six-way manifold which divides the sample into six equal samples, one for each individual channel of the test apparatus. These samples pass through the photochemical sensor units (PSU) where the effluent-biosensor reaction occurs.

The exhaust ports of the six PSU's are connected to individual flowmeters which allow the independent adjustment of flow rate for each channel over the range of 0.5 - 5 liters/minute. The six flowmeters are connected in parallel, via a second six-way manifold, through an integral

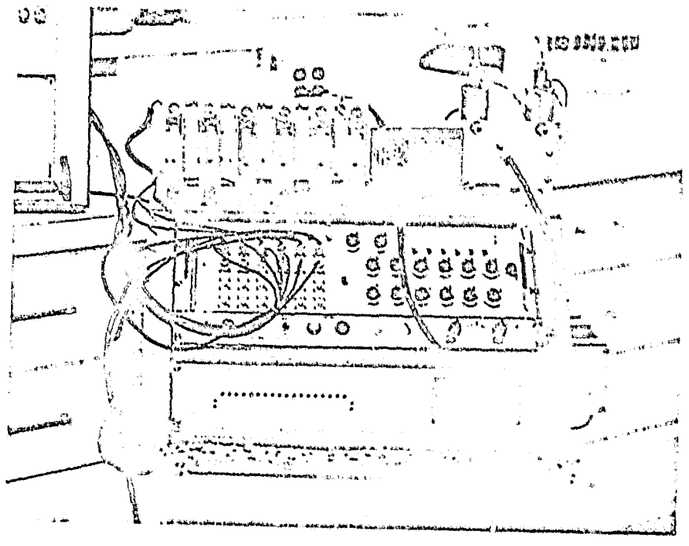


Figure 1. Front View of Laboratory Test Apparatus

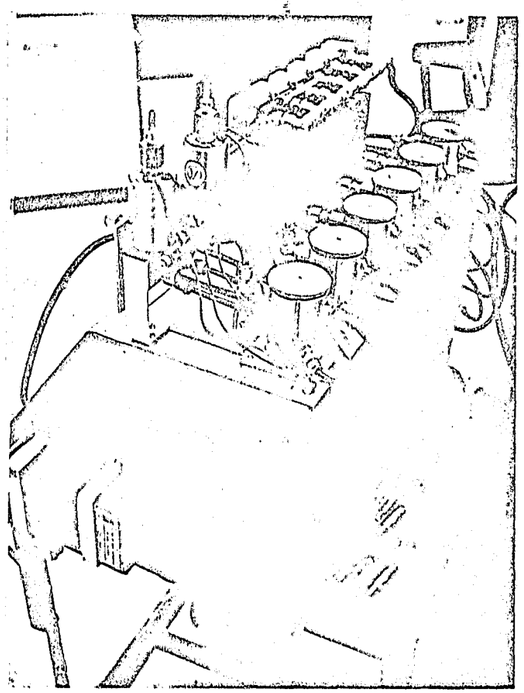


Figure 2. Rear View of Laboratory Test Apparatus Showing the Six Photochemical Sensor Units

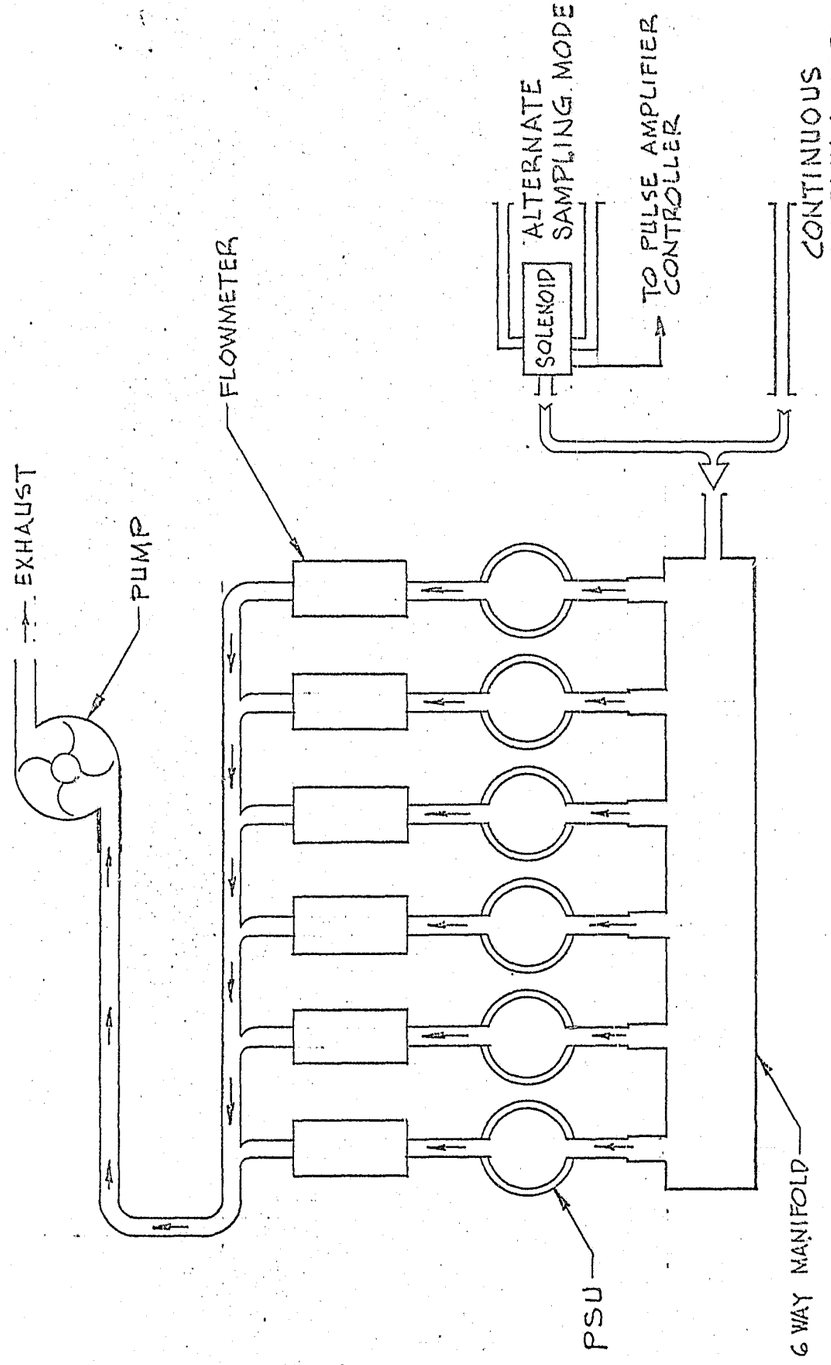


FIG. 3 GAS SYSTEM FUNCTIONAL FLOW DIAGRAM, LABORATORY TEST APPARATUS.

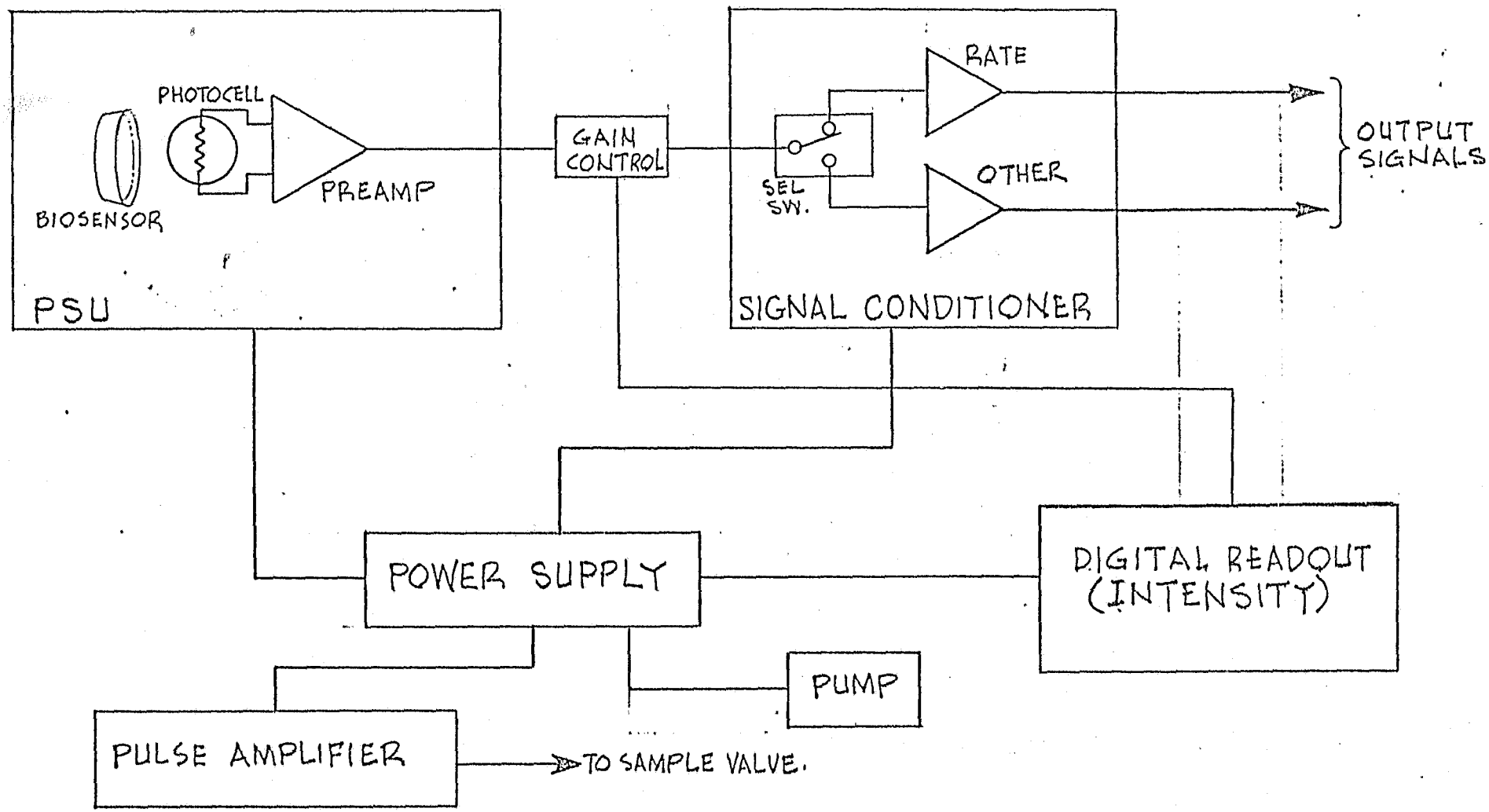


FIG. 4 ELECTRONIC SIGNAL PROCESSING FUNCTIONAL DIAGRAM, LABORATORY TEST APPARATUS.

pump, and then to an exhaust port. The integral pump limits the maximum flow rate through each channel to 3 liters/minute. Faster flow rates are possible by connection of an external, high capacity pump. This continuous sampling method is the simplest, and most often used mode.

As an option, the gas sampling system may be connected in an alternate sampling mode. In this configuration the sampling of the effluent may automatically alternate between two different sources. Switching is accomplished via a 3-way electrically actuated solenoid valve controlled by the pulse amplifier-controller circuit. Sampling rate and duration may be individually controlled. Generally, one probe (the reference or background probe) samples laboratory or control air as a reference, and the second, or sampling probe, samples the test effluent. This is the method of choice for sampling either effluents containing toxic compounds or environments in which effluents of interest may be continually and gradually increasing or decreasing in concentration.

Additionally, the alternate sampling mode may be used in a manual mode. In this configuration, the sample probe remains in the test effluent and sampling occurs manually on demand by push button. In either of these alternate sampling modes the sampling time duration is automatically limited to the desired interval by the pulse amplifier-controller circuits. This method allows accurate and repeatable control of sampling time.

Hardware utilized in the gas sampling system is constructed, where possible, of inert materials, generally stainless steel and nylon. Fittings provided at all connection points in the system, allow easy conversion of the apparatus between the various modes of gas sampling.

Photochemical Sensor Units

The term photochemical sensor unit or PSU refers to that subassembly which is composed of an exposure chamber, a biosensor holder, a lens system, a photosensor, inlet and exhaust ports, and an integral pre-amplifier circuit. The PSU interfaces optically with the biosensor surface and the photosensor; gaseously with the sampling network; and electronically with the signal processing circuitry. The exposure chamber design considers flow rate, port configuration, lens system, and biosensor holder. The laboratory test apparatus consists of six PSU's, each individually controlled.

The integral preamplifier circuit and the photosensor provide an output signal (to the signal processing circuits) which is proportional to the intensity illuminating the photocell. The light output of the biosensor is focused on the photosensor (photocell) by a lens. Variations in the light level (intensity) of various biosensors are accommodated by individual gain control circuits. The biosensor, which is housed in a petri dish, is inserted into the PSU holder, which in turn couples the biosensor to both the photosensor circuitry and the gas sampling network.

Electronic Circuitry

The electronic circuitry (exclusive of the preamplifier circuit described in conjunction with the PSU) consists of signal conditioner circuits, pulse amplifier and control circuits, intensity readout, signal outputs, and power supplies.

The signal conditioner circuits selectively amplify the output signal of the photochemical sensor unit. The circuit designs consider amplitude,

waveshape, and timing of the input signals and provide output signals, compatible with recording instruments, proportionate to a mathematical function of the input signal (the change in luminous intensity of the biosensor). A total of 18 signal conditioner circuits, six sets of 3 types each, are included in the laboratory test apparatus. The three integral types of signal conditioner circuits (rate, rate-reset, and DC-offset) are incorporated to provide useful information output from the variety of possible input signal waveshapes, amplitudes, and frequencies.

Sampling valves included in the gas sampling system are controlled by pulse amplifier circuits. Operation of these sampling valves is possible in both an automatic and a manual mode. In the automatic mode, the valves open and close for a preset time and at a preset interval. Both "on-time" and repetition rate are individually controlled. In the manual mode, the valve either opens for a preset time on demand (activation of a push button) or remains open for the interval of time during which the push button is activated. Additionally, the pulse amplifier circuits provide output signals to indicate sample times on recording equipment.

An intensity readout circuit, incorporating a digital voltmeter, is included in the apparatus. The circuit is connected, via a selector switch, to each PSU and in conjunction with the individual gain control circuits provides an accurate, repeatable method of adjusting the nominal intensity of biosensors to equivalent settings. This assures accurate comparisons between biosensors in the individual PSU's. An intensity output signal is available for those circumstances where a record of intensity is necessary.

Access to the output signals of the various signal conditioner circuits, and the additional pulse amplifier and intensity outputs mentioned above,

is provided by a series of standard output jacks. These outputs, connected to strip chart or magnetic tape recorders, provide permanent records of tests conducted utilizing the test apparatus. Output signals for each PSU, and for each signal conditioner circuit are available. It is also possible to connect the preamplifier outputs to external signal conditioning circuitry, should this be desired.

Internal power supplies provide proper polarity and amplitude operating potentials to the various subassemblies of the test apparatus. Included are line voltage for the pump and intensity readout circuits, + and - 15 VDC for signal conditioner circuits, and 28 VDC for the sampling valves.

BACKGROUND TESTING

Detection, utilizing bioluminescence, is based on the ability of the biosensors to react to various components of their gaseous environment. Past experience with bioluminescent detection programs has shown that many biosensors are sensitive to a large variety of gases. This reactive diversity requires control. A given biosensor, sensitive to the vapors of interest (in this case, those of heroin and marijuana) under controlled conditions must also be capable of detections in the field where the effluent of interest is mixed with many additional effluents, rather than with relatively clean laboratory air. Background studies were conducted as part of this program to study the environment in which detections of narcotic effluents will ultimately be made. The background tests were conducted over an 11 day period in August of 1970 in New York City.

Tests were conducted utilizing two RPC Corporation prototype detector units. These units differ principally in gas sampling rate and PSU exposure chamber design.

Two different types of detectors were utilized both to increase the validity of the tests and to study the response of the biosensors utilized under varying conditions. Both flow rate and exposure chamber configuration have been shown to affect sensitivity, and studies of the effects of these parameters are therefore necessary.

Two different strains of biosensors were utilized in the background tests. Both had previously been shown to have sensitivity to heroin and marijuana under controlled conditions. For each sensor strain, tests were

conducted on two media formulations, at a minimum of two ages, in each detector unit.

Sites for testing were selected by members of the Narcotics Division, New York Police Department in conjunction with RPC Corporation test personnel. All sites had a past or present history of narcotics use and/or sales problems. A cross-section of indoor and outdoor, residential, commercial, and public sites were visited; location included Spanish and Negro Harlem, lower midtown Manhattan, Greenwich and East Villages, Queens, the East River waterfront, and J.F.K. Airport. When possible, sites were tested (as described below) both in morning and afternoon or evening hours. In general, samplings at each site continued for a minimum of ½ hour actual testing time.

All test equipment utilized was powered by self-contained batteries with the exception of the strip chart recorder. This recorder (Techni-Rite Electronics TR-711), used to provide a permanent record of the testing, requires 117 V A.C., and consequently was powered by a portable power pack, consisting of rechargeable 15 VDC batteries and a converter, which provided the necessary operating voltage and current. Use of this power pack and self-powered detectors eliminated dependency on wall outlets and allowed testing at various remote and/or outdoor sites.

At each site, a record of the gas sampling was made utilizing both detectors, and the various ages and types of biosensors. Records of each site include date and time of day, temperature, location, detector, biosensor type and age, strip chart speed and sensitivity. In addition, notes include a general description of the environment sampled. Examples include instances where certain potentially interfering effluents were noted. Examples of these include coffee (dock warehouse), garlic cooking

odors (Spanish Harlem), fuel oil (various sites), human excrement (tenements), partially decomposed bedding (basement shooting gallery) and musty conditions (basement apartments). Additionally, samplings were made at such diverse locales as: abandoned, partially burned-out tenements used as shooting galleries; garbage containers; clothes closets and dressers; fish and food markets; bathrooms and kitchens; and hallways, stairways, and roof landings. It should be emphasized that the decision to visit sites more than once, though desirable, was not always possible. The Narcotics Division personnel assisting the RPC Corporation team felt that returning to some sites would cause too much concern and commotion with residents.

Several representative sample strip charts of background testing are shown in Figure 5. Note should be made of the quiet background (normal gaseous environment, in which detections are desired, which does not produce spurious signals or oscillations). Backgrounds of this type, with low noise level (normal, small amplitude signal fluctuations occuring during steady-state samplings) are desirable as they simplify the problems of electronic signal or gas filtering.

During the course of the background studies, test personnel had the opportunity to sample several apartments used as heroin packaging factories. The testing proceeded in the manner previously described after the apartment was secured by detectives, but before the confiscated narcotics were removed. In these instances, heroin was detected utilizing the same detectors and biosensors used for the background studies. Again, samplings taken in the apartment, but away from the heroin yielded low noise background levels. Figure 6 is a sample section of strip chart showing background signals and heroin signals obtained under these

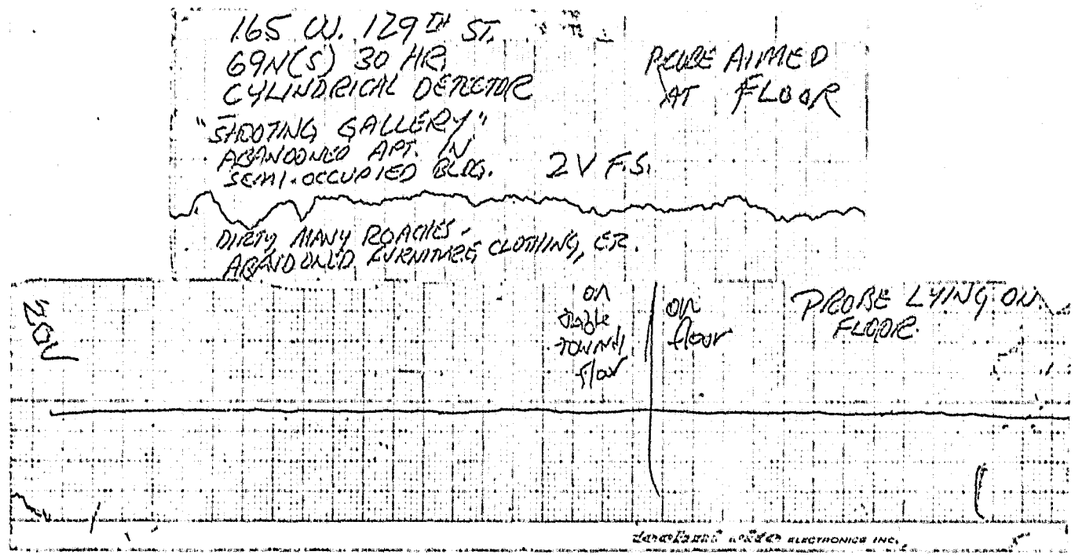


Figure 5. Samples of General Background Testing. From top to bottom they show representative recordings from a tenement, a dry goods stall in a public market, and a fish market. For all samples, chart speed was 1 mm./sec. and sensitivity was 1 volt/division (except the top sample which is 100 mv/division).

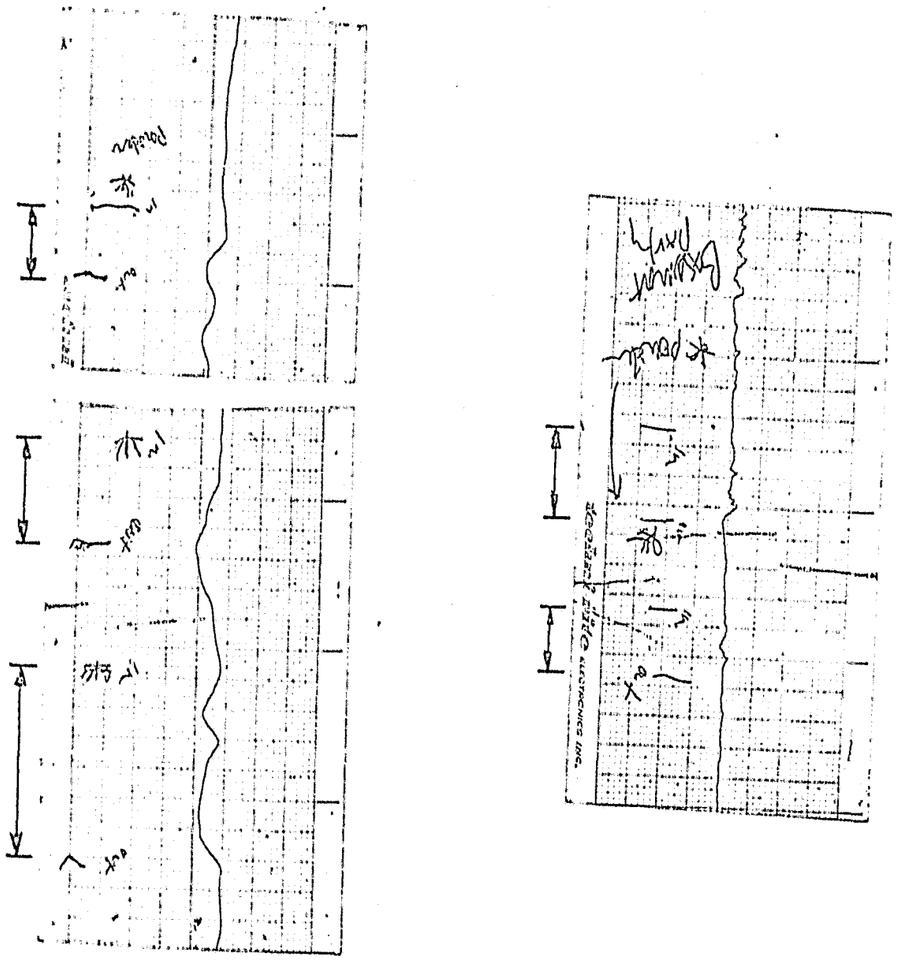


Figure 6. Strip Chart Recordings Showing General Background Sampling and Detections of a Powder Subsequently Shown (by Police Laboratory analysis) to be 27% Heroin. The recordings were made using the same biosensor in two different detectors. For both samples chart speed was 1 mm./sec. and sensitivity was 1 volt/division. Sampling intervals are indicated to the right of each chart.

circumstances. The heroin signal obtained was of the same general wave-shape, polarity, and amplitude as those obtained in the laboratory with the same biosensor. Several repetitions of this detection were made for verification.

The data collected (strip chart recordings) were analyzed for the presence of potentially interfering or masking effluent components present in the atmosphere, which might interfere with the ultimate detection of narcotics. Potential interference would be indicated where the gaseous environment contained effluents which may yield signals which closely resemble those of heroin or marijuana. Additional potentially interfering effluents are those where the biosensor response oscillates in such a manner as to mask detections of narcotics. No instances of problems of this nature were encountered in any of the sites tested.

LABORATORY TESTS

Although over a hundred sensor strains were available at RPC Corporation at the start of this program, they had not all been evaluated for sensitivity to the specific requirements of this program. These strains, and new isolates from nature, had to be screened to allow selection of potentially useful strains. Additionally, it was expected that modifications of the selected strains would be desirable as a method of providing sensors with more useful characteristics. These two tasks, screening and strain selection and development, form the nucleus of the laboratory test programs (See Figure 7).

Screening

Screening refers to the systematic testing of the biosensor strains to determine their response, if any, to the test chemicals of interest (heroin and marijuana). Initially, screening is performed to survey a collection of strains and select those capable of generating repeatable signals. Ultimately, these potentially useful strains, and any new isolates or mutants found, are screened repeatedly to check the effects of such variables as temperature, relative humidity, or nutritional composition of the culture medium.

Preliminary screening

Most screening was performed utilizing the integrated laboratory test apparatus which provided for comparison of 6 sensors simultaneously. This capability was utilized to compare simultaneously, the signals from identical or differing strains which have undergone a variety of preparation

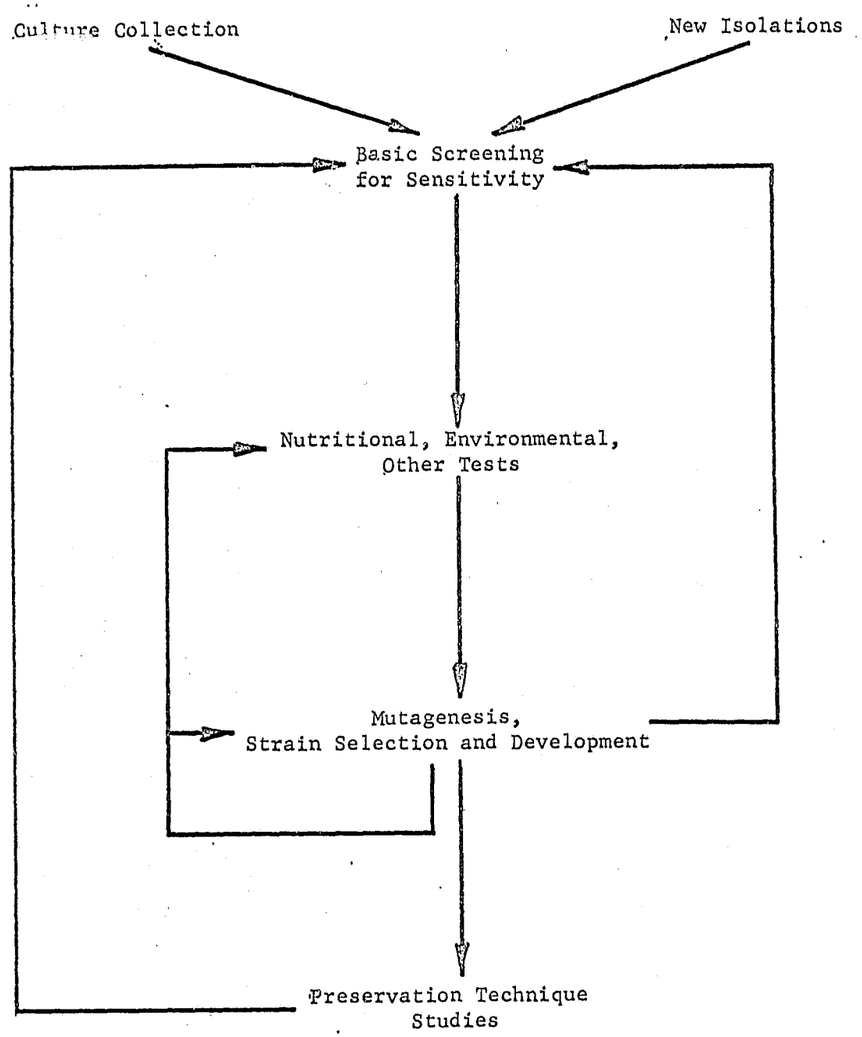


Figure 7. Basic Laboratory Testing Flow Diagram. Note continual re-evaluation of strains (to study nutritional requirements of mutants, for example).

schemes, prior to their utilization as sensors. The testing procedures are presented in the flow diagram (Figure 8). Briefly, the procedure involved the comparison of signals resulting from controlled time and flow rate exposures to both heroin and marijuana effluents, and an equivalent exposure to a control container. The signals were recorded on a strip chart recorder (Brush-Clevite Mark 260). The sensors selected from the above task as potentially useful, were those generating repeatable signals upon exposure to effluents of marijuana and/or heroin.

Sensitivity of the sensors was checked at a minimum of two ages, 24 and 48 hours, and when possible at 72 hours after sensor inoculation. Sensors which did not generate signals to either effluent were not checked further. Sensors meeting the minimal criteria mentioned above were checked again to insure repeatability.

In addition to the strains of bacterial biosensors from the RPC Culture Collection, screenings of luminescent fungi and additional bacterial species obtained from the American Type Culture Collection were undertaken. New isolates were obtained from Japanese, Mexican, and domestic marine sources and were checked as part of this program.

These studies resulted in the selection of 17 potentially useful biosensors out of more than one hundred tested.

Tables 1 and 2 show the principal characteristics of these selected strains from the RPC Culture Collection, and the new isolations, respectively.

Effects of biosensor age

Response to specific environmental changes is conditional, although the ability to respond in a specific manner is genetically controlled.

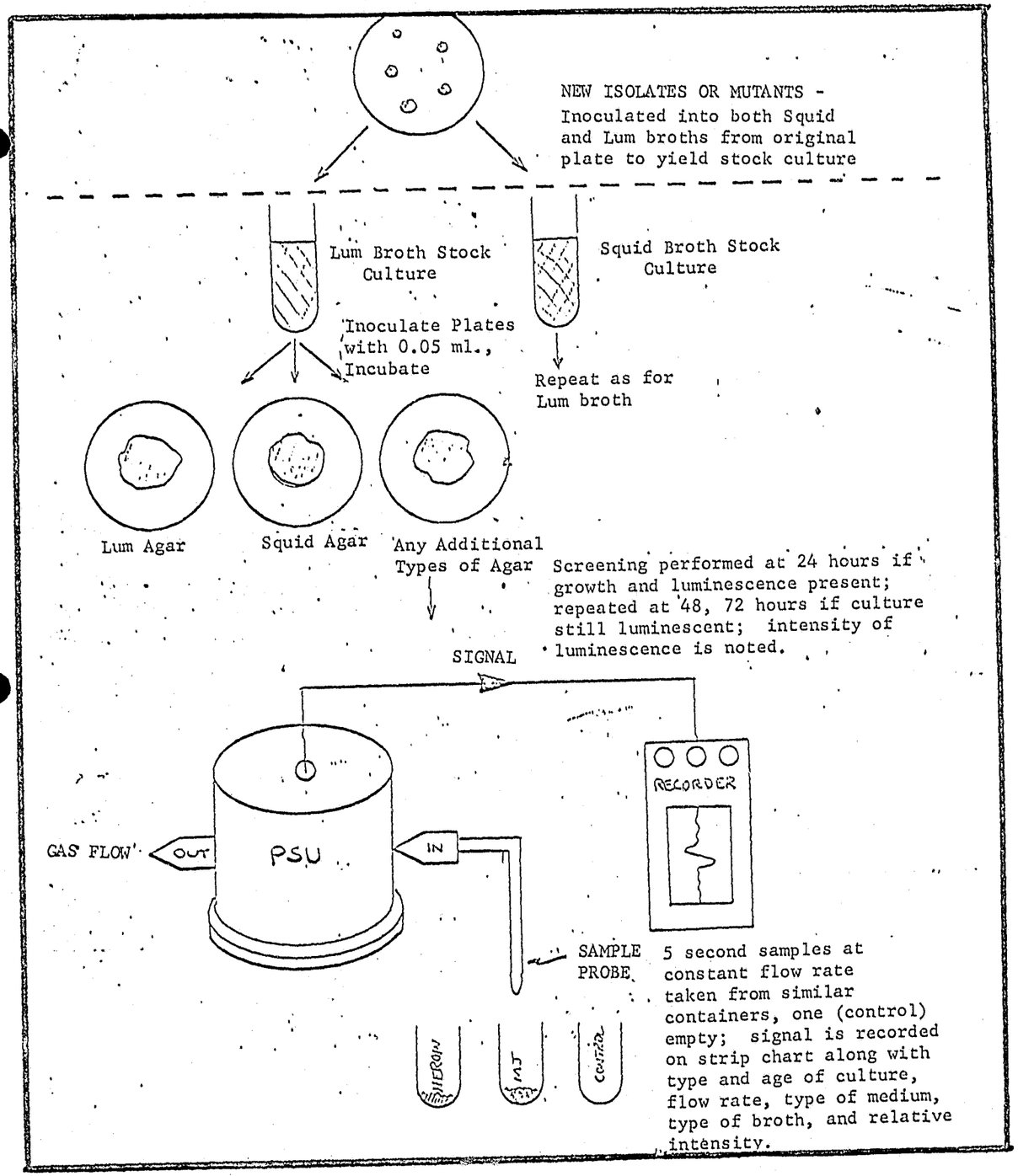


Figure 8. Flow Chart Showing Standard Screening Procedures

BIOSENSOR	AGE, HOURS	CULTURAL AND SIGNAL PARAMETERS			
		LUM/LUM[1]	SQ/LUM	SQ/SQ	LUM/SQ
C4/333	24 48	+MJ +H[2] NL[3]	+MJ +H NL	+MJ +H NL	+MJ +H NL
C4/336	24 48	+MJ +H -MJ +H	+MJ +H NL	+MJ +H NL	+MJ +H NL
C4/345	24, 48	+MJ +H	+MJ +H	+MJ +H	+MJ +H
C4X	24, 48	+MJ +H	+MJ +H	+MJ +H	+MJ +H
C4Z	24 48	+MJ +H +MJ θH[4]	+MJ +H +MJ θH	+MJ +H +MJ θH	+MJ +H +MJ θH
C5MJ	24 48	+MJ -H θMJ -H	+,-MJ -H θMJ -H	-MJ -H +MJ θH	-MJ -H +MJ θH
C9	24, 48	+MJ -H	+MJ -H	NG[5]	NG
C10	24	+MJ +,-H	NG	NG	+MJ +,-H
C11	24 48	-MJ -H -MJ -H	-MJ -H -MJ -H	-MJ -H -MJ -H	-MJ -H -MJ -H
C30	24 48	+MJ +H +MJ +H	+MJ +,-H +MJ +H	+MJ θH -MJ -H	+MJ -H -MJ θH
C43	24 48	θMJ -H +MJ -H	θMJ -H +MJ -H	θMJ -H θMJ -,+H	θMJ -H NL
C57	24, 48	+MJ +H	+MJ +H	+MJ +H	+MJ +H

TABLE 1, Principal Characteristics of Biosensors Selected from the RPC Corporation Culture Collection.

1) Culture designations indicate source of inoculum and type of sample matrix. LUM/SQ indicates LUM Broth Culture inoculated onto squid agar.
2) Indicates polarity of signal
3) NL indicates no luminescence
4) θ indicates no sensitivity
5) NG indicates no growth

BIOSENSOR	SOURCE	AGE	CULTURAL AND SIGNAL PARAMETERS		
			LUM/LUM[1]	SQ/SQ	LUM/SQ
62N	JAPAN	24, 48	+MJ +H[2] θMJ[3] +H	+MJ -H θMJ +H	+MJ -H θMJ +H
69N	JAPAN	24, 48	+MJ +H	NL[4]	-MJ -H
P_2^N	JAPAN	24, 48	+MJ +H +MJ +H +MJ θH	+MJ +H NL	+MJ +H +MJ +H +MJ θH
C50	SO. CALIF.	24, 48 72			
C67	SO. CALIF.	24, 48			

TABLE 2. Principal Characteristics of Biosensors - New Isolates

1) Indicates source of inoculum and type of sample matrix (See Note 1, Table I)
2) Indicates polarity of signal
3) θ indicates no sensitivity
4) NL indicates no luminescence

Conditions affecting the response include (among others discussed below) the age after inoculation of the biosensor culture. Experience has shown that strains may, for example, show no sensitivity (no response) at 24 hours (age) while showing good sensitivity at 48 hours. The reverse is also possible.

Because of this, preliminary screenings of strains, regardless of source, were conducted at 24 and 48 hours, as a minimum. This provided assurance that potentially useful strains would not be overlooked. It should be mentioned that this dependency on age should not be construed as indicative of the ultimate useful life span of a biosensor, as this is dependent on additional factors, including nutrition. An objective of strain selection and development is, among others, an increased life span for biosensors.

It should also be mentioned that this multiple age screening was also used, as a normal procedure, for all mutant screenings, nutrient evaluations, and other studies on the effects of a given parameter.

Effects of temperature and relative humidity

Both temperature and relative humidity are factors affecting the detection of narcotics utilizing the bioluminescence concept. Parameters affected include the sensitivity and useful life span of a biosensor, and the amount of vapors evolving from a source.

These effects were investigated with the biosensor, the effluent source, or both at controlled environmental parameters provided by a HotPack Environmental Chamber. Parameters utilized were 10°C, 30% R.H., 20°C, 50 and 70% R.H., and 35°C, 25 and 80% R.H.

Results indicate that 20°C, 70% R.H., are optimal biosensor

environmental parameters, and this is consistent with past experience. Higher temperatures, or lower humidities (in the range tested) allow detections, but significantly shorten the useful life span of the biosensor. Lower temperatures, as would be expected, significantly reduce sensitivity.

Additional tests (see Feasibility Demonstration Section) were conducted with the biosensor environment fixed at the optimum parameters, but with effluent source both warmer and colder, with various R.H. levels.

Problems were encountered with these methods only when rapid (2-4 times per hour) fluctuations of relative humidity occurred. No stable R.H. level presented any problems, signal changes, or signal attenuation. Analyses have shown that the problems encountered were due to the R.H. change in the sample and control containers lagging behind the R.H. change in the environment. The problem was due to the screening method used, rather than the biosensor system itself. Problems of this nature would not be expected in the field where the environment containing narcotics or narcotics packages would be sampled.

The tests showed that detections of heroin utilizing the bioluminescence concept are possible when the effluent source and/or biosensor are at a variety of temperature and R.H. levels, but that optimal detections require maintenance of the biosensor at or near 20°C, with a relative humidity of 50 - 100%. Present sensitivities also require that effluent concentrations be at least equivalent to that evolving from the sources tested (see Feasibility Demonstration Section). Detections of narcotics effluent sources at temperatures lower than approximately 15°C require either greater source surface to volume ratios, larger source volumes,

biosensors with increased sensitivity, or a combination of these. This is necessitated by the lowering of vapor pressures with temperature.

Strain Selection and Development

Strain selection and development refers to a program of studies directed at the production of biosensor strains with more desirable characteristics, such as better sensitivity or longer life. Strains to be studied in any of the strain selection program tasks are those which prior screening and tests have shown to be sensitive to heroin and/or marijuana. The program involves the systematic mutagenesis and testing of selected strains, utilizing varied media formulations and nutritional additives. Details and results of both the varied forms of the screening and the strain selection and development programs are discussed below. Formulae for all nutrient media are included in the Appendix.

Ultraviolet irradiation mutagenesis

All twelve (12) candidate biosensors have been subjected to a minimum of one set of ultraviolet irradiation (UV) parameters. The basic procedure is shown diagramatically in Figure 9. Briefly, the procedure involved the exposure of a known population of cells to a standard dose of UV for several time intervals, with periodic sample removal after 1, 5, and 15 seconds exposure. These samples were inoculated (plated) onto nutrient agar, incubated and checked for evidence of mutagenesis. The majority of cells were killed by the exposure to UV; furthermore, the majority of mutants were lethal. Survivors of the irradiation were checked for qualitative evidence of mutagenesis. Factors considered were those affecting both physiological characteristics (bioluminescence, growth patterns) and

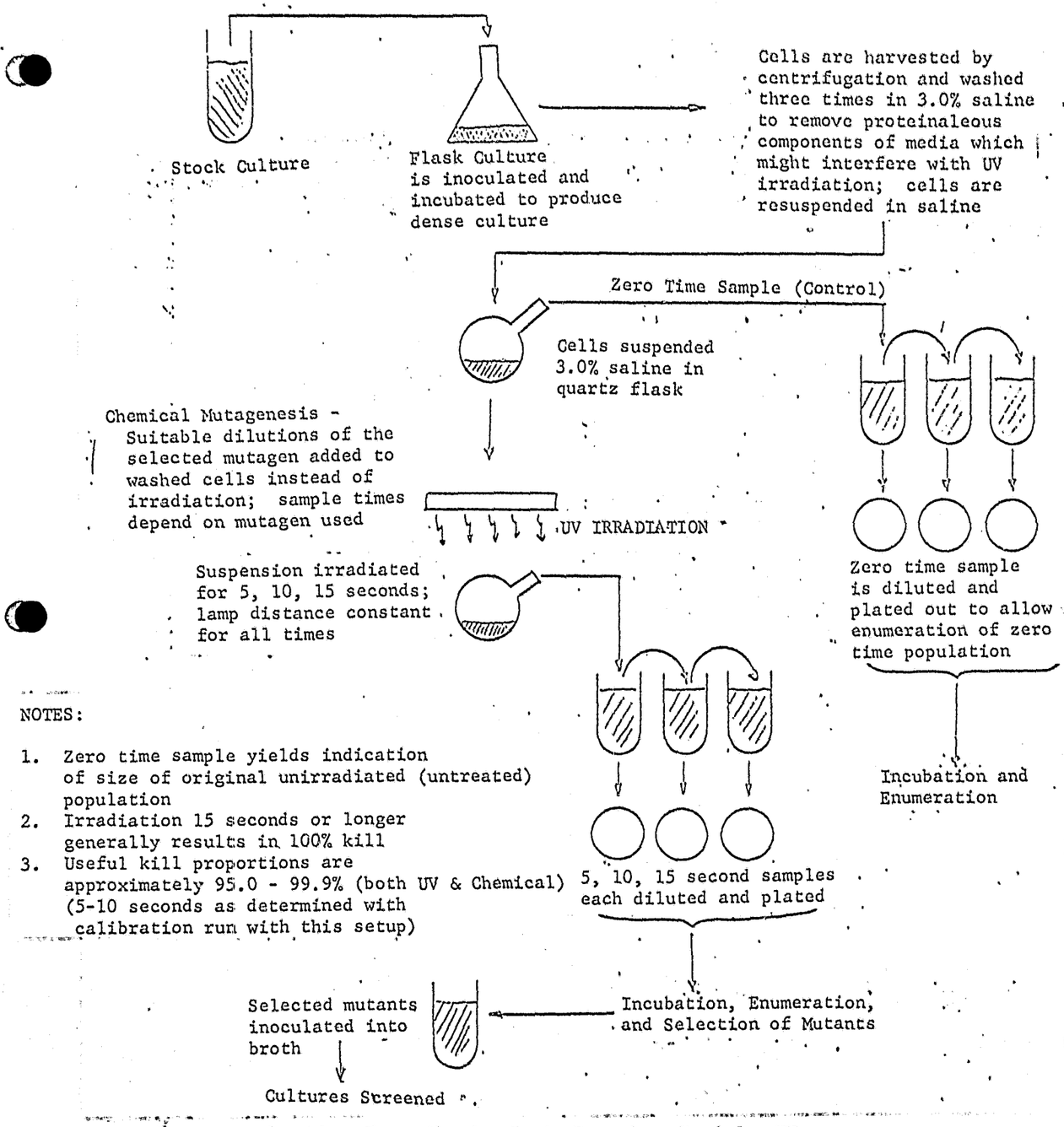


Figure 9. Flow Chart Showing Basic Procedure Used for UV and Chemical Mutagenesis

morphological characteristics (colony size, shape, color). Evidence of changes in any of these characteristics, in comparison to the control (parent) strain was considered indicative of effective mutagenesis. These colonies were selected, inoculated into a nutrient broth (liquid) growth medium, incubated and subsequently screened for sensitivity in the manner described above.

There were definite probabilities that a proportion of the mutants would, and a proportion would not, retain the sensitivity to narcotics exhibited by the parent strains. Furthermore, there were definite probabilities that among those mutants which did retain sensitivity, some proportion would have decreased sensitivity. Only mutants demonstrating increased sensitivity would be retained and checked further.

A total of 23 UV mutants were isolated and screened for sensitivity to narcotics. Parent strains of all mutants were one of the twelve original candidate biosensors or one of the five new isolate candidate biosensors as listed in Tables 1 and 2 respectively.

Three isolates of strain C-10, out of 13 selected, showed significantly increased sensitivity to narcotics in comparison to the parent strains. These strains - LC-10A, LC-10D, LC-10F - were screened in detail to determine optimum age and nutrient medium formulations, and reliability. Analysis of the complete data showed that only LC-10A was capable of yielding repeatable signals. It was, therefore, subjected to additional irradiation (and chemical) mutagenesis experiments in an attempt to isolate mutants with still increased sensitivity. No isolates with these characteristics were, however, isolated from the re-irradiation of LC-10A.

Additionally, UV irradiation isolates resulted only from strains C-4X,

C-9, C-10 and C-50. All were screened in the manner previously described. No mutants demonstrating increased sensitivity were isolated.

An additional technique, described in the literature, was investigated. This is based on evidence that the addition of caffeine to the nutrient agar used for the isolation of UV mutants will retard the normally occurring repair of irradiated genes. Other than this addition of caffeine, the procedure was the same as that previously described. The method was tested with C-4X and C-9. No useful mutants were isolated with these techniques out of 33 mutants isolated.

Further testing, at various ages and with various nutrient agar formulations established that none of the UV mutants were capable of consistent narcotics detection and they were not utilized for further studies.

Chemical mutagenesis

Four chemical mutagens were selected, based on both known efficacy and availability. These were 5-Bromouracil (5BU), 2-Amino purine (2AP), Acridine Orange (AO), and N-methyl-N'-nitro-N-nitrosoguanidine (NG). The procedure for chemical mutagenesis and subsequent screening is basically similar to that for UV mutagenesis, with the principal difference being the mutagenic agent. Reference should be made to the preceeding section and Figure 9 for procedural details.

Six biosensors were subjected to mutagenesis using the four chemical mutagens at a minimum of 4 exposure times (5, 10, 15, and 30 seconds). Isolates resulting from these procedures (see Table 3) were screened

		MUTAGEN			
		AO	5BU	2AP	NG
CULTURE	C4/336	16	2	2	9
	C10A	0	3	0	8
	C11	0	7	0	0
	C43	0	0	2	0
	C50	0	0	4	6
	P_2N	0	0	0	2

Table 3. Matrix Showing Origin and Number of Isolates From Chemical Mutagenesis Experiments. AO indicates Acridine Orange, 5BU indicates 5-Bromouracil, 2AP indicates 2-Aminopurine, NG indicates N-methyl-N'-nitro-N-nitrosoguanidine, 0 indicates no mutants isolated.

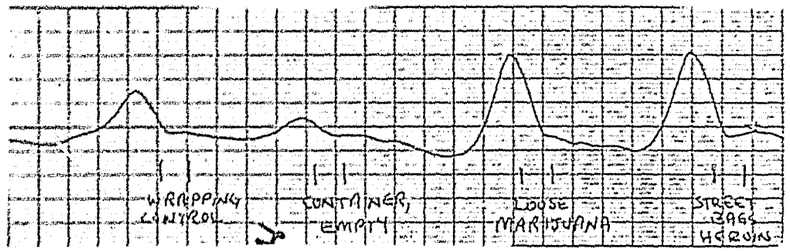
for sensitivity in the manner previously described. Several promising mutants of C-50 were isolated from the Nitrosoguanidine treatment and were subjected to detailed screening. Additional mutants with increased sensitivity were not isolated.

Strain LC-50XYZ, an NG mutant of C-50, was shown to be the most sensitive strain for heroin detection. The parent strain (C-50), a new isolate from a locally obtained squid, is somewhat less sensitive but more stable. LC-50XYZ, however, with some additional work aimed at improving consistency, might prove to be the best heroin detection strain. Figure 10 is a sample of results showing the comparison between parent and mutant strain.

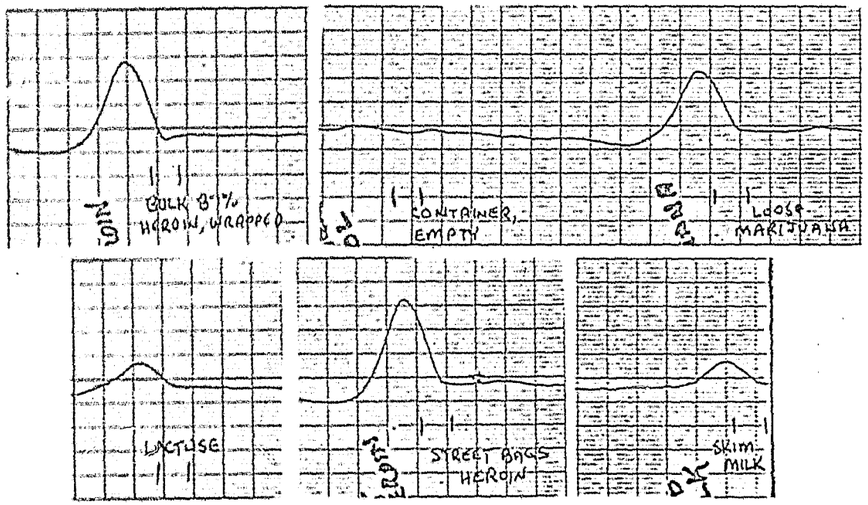
Ultrasonic mutagenesis

The use of ultrasound (20KHz) as a mutagen has been reported in the literature. This technique was tested utilizing C-50, the most sensitive, consistent, and reliable biosensor strain. The technique, basically similar to that used for UV and chemical mutagenesis, involves exposure, for varied times, of known density saline cell suspensions to the ultrasound. As with other mutagenic techniques, the test suspension and a control suspension are plated out, incubated, and checked for evidence of mutagenesis.

These trials, all involving C-50 but with widely varied times of exposure and/or power levels, have shown that the strain tested is particularly sensitive to cell disruption by sound. Minimal exposure time/power level tests showed no evidence of population reduction or mutagenesis, while only slightly longer exposure times or higher power



C-50XYZ



C-50

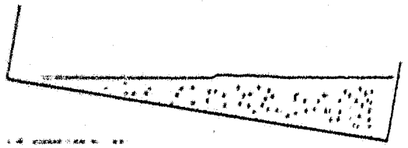
Figure 10. Sample Strip Chart Recordings Showing Comparative Reactivity of Mutant and Parent Strains of C-50. Chart speed was 1 mm./sec. and recorder sensitivity was 500 mv/div. For the samples shown, both biosensors were prepared on Squid agar from Squid broth, but C-50 was 24 hours old, while C-50XYZ was 72 hours old. At 72 hours, C-50 was nonreactive to all narcotics and controls, while C-50XYZ (as shown) retained the sensitivity it had at 24 hours. Wrapping control is newspaper, plastic, and paper tape as used to wrap bulk heroin. Although not shown in this figure, reactions of C-50XYZ, 72 hours, to other narcotics and controls was equal to, or superior to that of C-50, 24 hours. Sample intervals are indicated by the vertical marks below the pen trace.

levels resulted in 100% cell disruption. Therefore, this technique was not utilized further with this or other strains of biosensors.

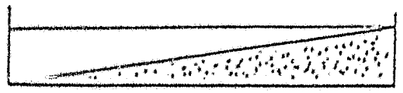
Drug dependency studies

The dependency of luminescence and sensitivity to the narcotics of interest was studied with twelve strains, with an aim toward characterization of a culture whose luminescence is heroin or marijuana-dependent. The procedures used involved the isolation and characterization of strains whose sensitivity to marijuana and heroin is dependent on the presence of these drugs in the nutrient matrix (agar). Initial investigations were performed using a gradient plate technique. This technique involved the use of a large container (plate) of nutrient agar prepared with two distinct layers (see Figure 11). The layers differed only in that one contained an additional component - the drug. The plate was prepared in two stages; the bottom layer was poured with the container slanted to provide the gradient; the top layer was poured, with the container level, after the bottom had solidified. The complete plate was allowed to remain overnight, thus allowing diffusion of the drug molecules between the layers and producing the gradient indicated. Strains of biosensors were inoculated in a uniform linear pattern across the gradient and incubated. Analysis involves the observation of the dependency of growth and/or luminescence on the drug at points proportionate to 0 - 100% of the concentration added.

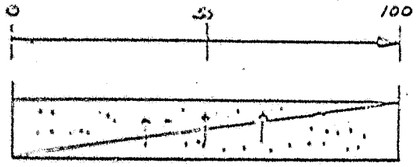
Additional investigation utilized a replica plate technique (see Figure 12). This method allowed the carefully controlled comparison of biosensors to a definite concentration of narcotics. Strains were



Bottom layer of basic medium with additive is poured and solidified on a slant



Top layer of basic medium is poured with container level



Additive concentration, % of total

Plate is incubated prior to inoculation to allow diffusion of additive into top layer, producing linear concentration gradient across plate; inoculation is made across the plate parallel to gradient. Gradient results from diffusion of additive molecules into basic medium of varying depths at any point across gradient; i.e. at midpoint concentration is 50% total due to diffusion into equal volume of top layer

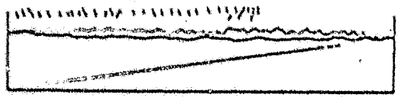


Plate after incubation - note presence of growth in presence of 0-100% of additive concentration, but luminescence only from 0-75% of additive concentration

Figure 11. Preparation and Use of Gradient Plates

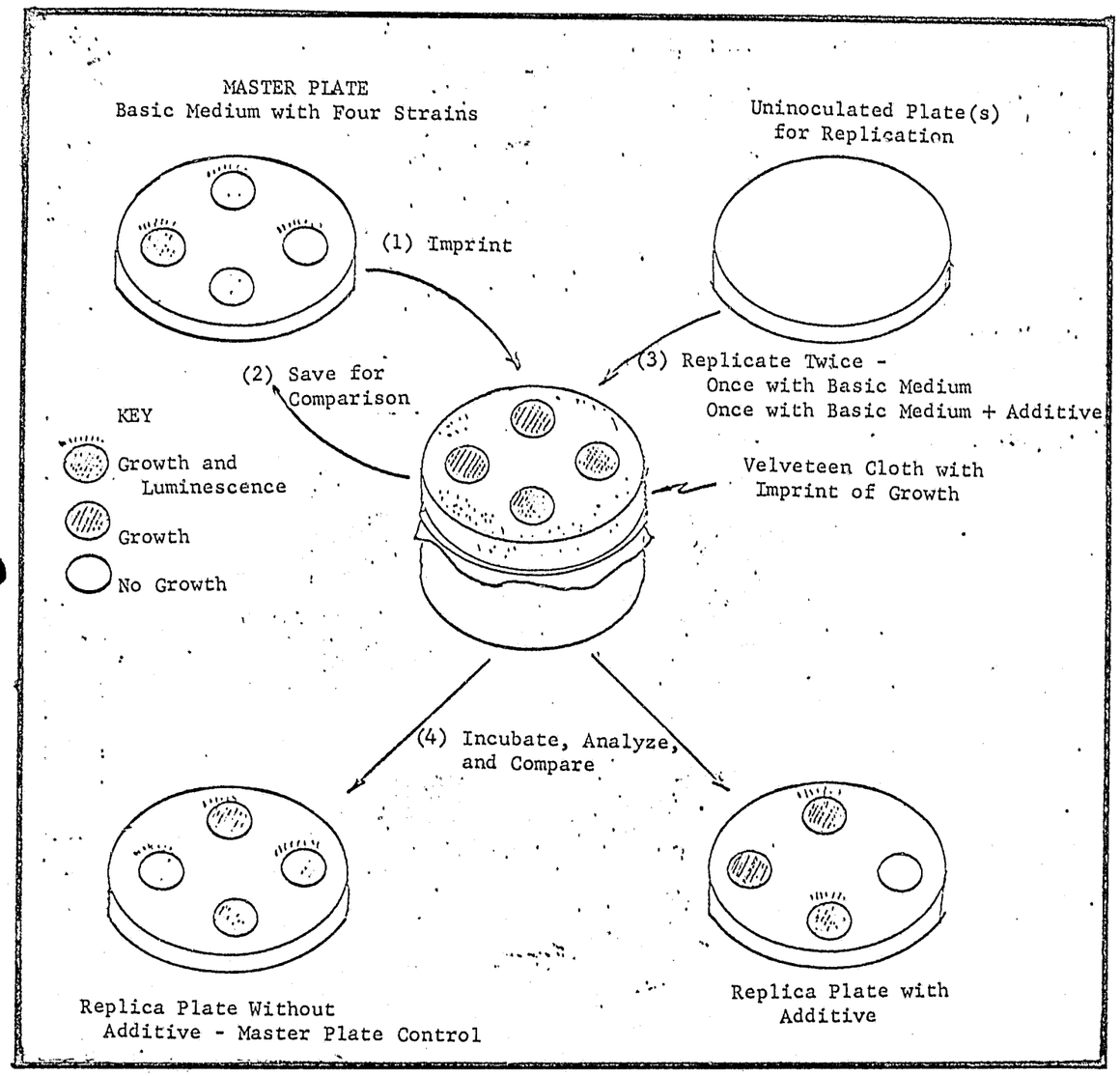


Figure 12. Method of Using Replica Plating

inoculated onto a standard nutrient medium in a grid pattern and allowed to incubate until good growth and luminescence were present. This grid pattern of several different biosensor strains was then simultaneously transferred to additional media, including a control medium (the same medium type as the original, or master plate). After appropriate incubation, comparisons were made between media, additives and strains as to both growth and luminescence (Figure 13).

The luminous intensity of several strains appeared, on initial investigation, to be heroin dependent. Strains C-4/345 and C-57 were significantly brighter on Lum + heroin than on standard (control) Lum. Strain C-50 was dimmer on Lum + heroin compared to the control Lum. C-11 showed varied results. Concurrent to a repeat of these tests, biosensors of these strains on Lum + heroin and standard Lum were prepared, incubated and screened for differences in sensitivity to heroin. These repeat tests and the screening showed that the apparent dependency on heroin was inconsistent and that no increased sensitivity to heroin resulted.

Additional drug dependency tests were conducted utilizing nutrient agar formulations prepared with both hot and cold marijuana extracts. A replica plate technique, as discussed above, was utilized. Ten strains tested showed uniformly dimmer luminescence in the presence of either the hot or cold marijuana extracts. No effect on sensitivity, however, was noted when several of these sensor strains were tested for sensitivity to narcotics.

The replica plate technique was also utilized to study the possibility that drug dependency might be induced if heroin were substituted for the normal carbon source (Glycerol) in Lum agar. This technique was tried

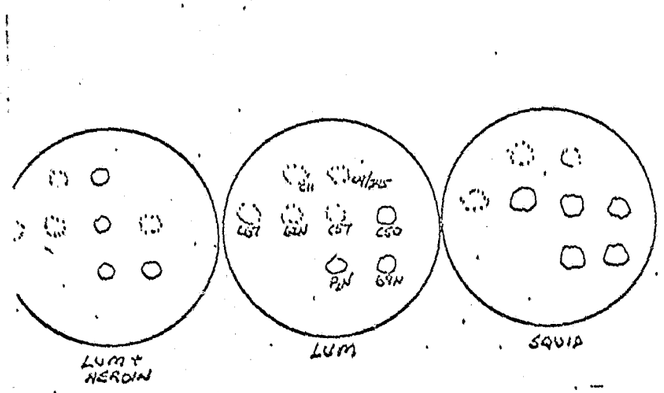


Figure 13. Photographic Record of a Series of Replica Plates Prepared from the Same Master Plate. Note that C57 and C4/345 are present but very dim on Lum Agar.

with strain C-11, which had previously shown evidence of inconsistent dependency on heroin for luminescence. However, no more consistent dependency was found utilizing this technique.

Inoculation geometry

The effect of inoculation geometry was studied with strain C-50, by preparing a set of biosensors which varied as to surface area inoculated, and thus surface to volume ratio. The broth volume used for all the plates was constant; all of the plates were prepared in one batch and inoculated from the same source. Analysis of the results showed that the normal inoculation pattern (0.05 ml. broth spread over a 20-22 mm. area in the center of the plate) was optimal in all respects for the sensor tested and the photosensor units utilized. This is consistent with other work of this nature, and verifies that the inoculation geometry utilized was optimal for the PSU configuration. Figure 14 is a tabulation of the results.

Pre-incubation of nutrient agar formulations

Pre-incubation of nutrient agar plates may be considered a method of adding components to the basic nutrient agar. The method involves the incubation of nutrient agar plates in a gaseous environment containing the desired compound. In the case described here, the environment was 100% oxygen. Plates were allowed to remain in the oxygen atmosphere for periods of 24-48 hours. The purpose of this pre-incubation was to provide higher oxygen concentrations for the biosensor since oxygen is necessary for luminescence and sensitivity. Formulations utilized for the study,

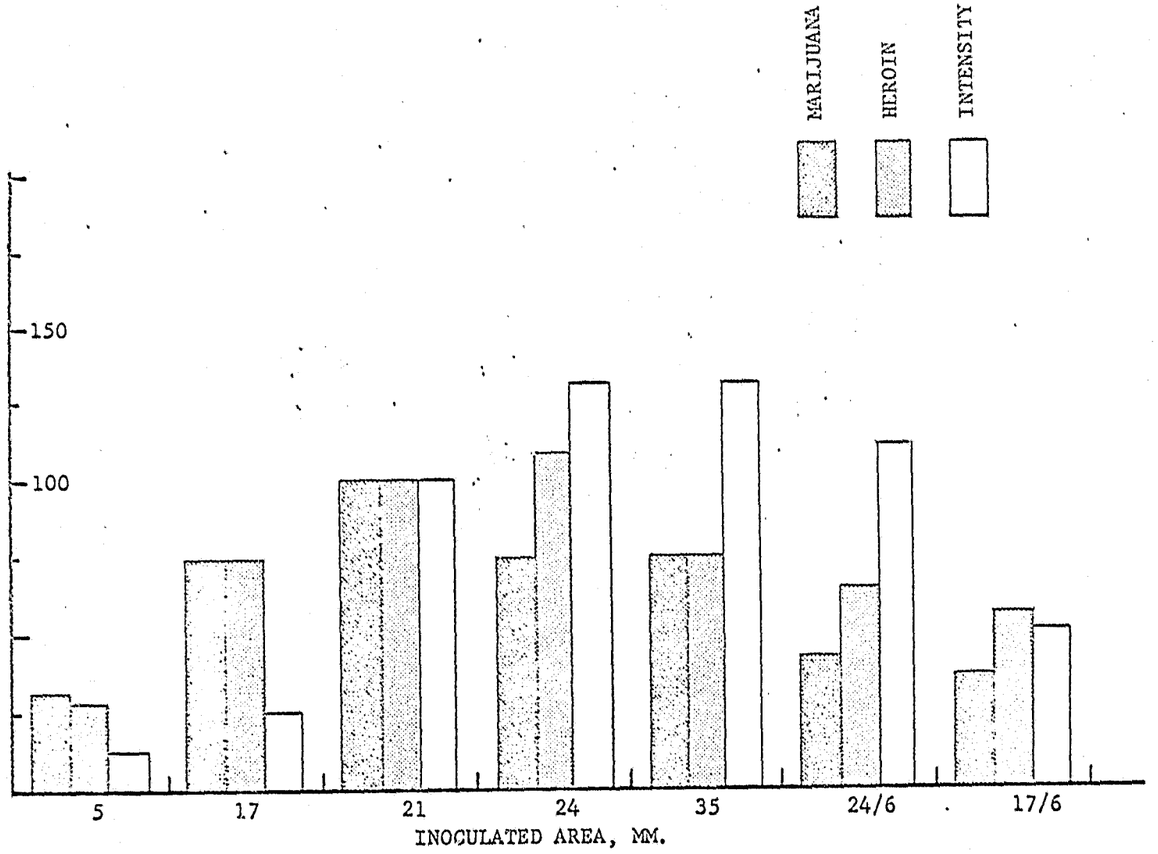


Figure 14. Effect of Inoculation Geometry on Reactivity. Strain C-50, Squid Broth to Squid Agar, 24 hrs. old was used for all tests. Sampling duration was 9 seconds and the normal screening procedures were used. The standard pattern was taken as the 100% mark, and the other values graphed based on this standard. Control is the 21 mm. area. The last two samples shown are concentric circles of inoculation, and the figures indicate outside and inside diameter of the inoculated area.

were both the standard Lum and Squid nutrient agar formulations and blood agar (a formulation containing 2.5% whole blood cells). Blood agar was used because of its hemoglobin content; an oxygen-binding molecule.

Results of these tests proved somewhat inconsistent, but in general the biosensors show increased sensitivity due to oxygen pre-incubation. Blood agar, however, was no more efficient than Lum or Squid agar with the same pre-incubation history. Figure 15 is a tabulation of the results.

Nutritional evaluations

Growth, luminescence, and sensitivity of microbial biosensors is dependent on the nutrient matrix used for their growth. Although capable of growth and luminescence on media of diverse nature, sensitivity to a given effluent may be affected by the formulation utilized even though growth and luminescence are not affected. To evaluate this, various nutrient formulations, both liquid for culture maintenance and solid for biosensor preparation, were utilized with the various strains selected. It should be emphasized that sensitivity testing on various nutrient agar formulations, as well as the other phases of the strain selection and development tasks, were repeated during the course of the program. These repeat trials were performed to verify test results, and to check response of mutant or new isolate strains as they became available.

Media formulations tested, listed in Table 4, were representative of the diverse types utilized in prior programs at RPC Corporation or listed in the literature. All had previously been utilized as growth

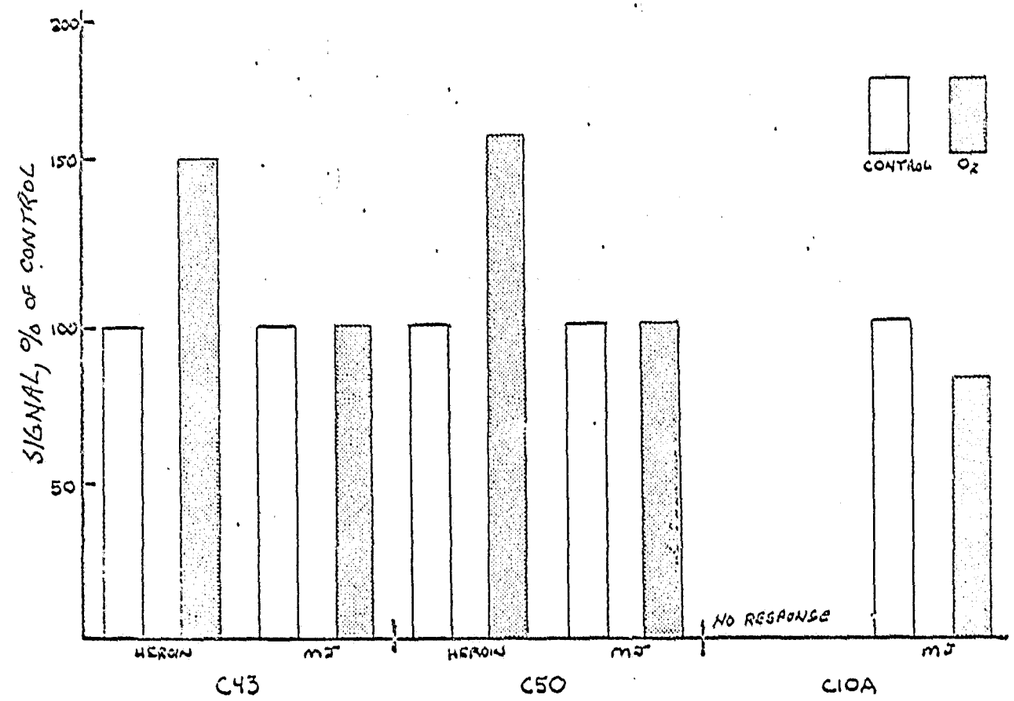


Figure 15. Effects of Oxygen Pre-incubation on Reactivity. In each case shown the nutrient agar plate control signal was taken as the 100% standard and the test signals graphed based on this standard. Control plates and pre-incubated plates were all from the same batch.

Luminous Agar (Lum)	Veal Infusion Agar (VIA)
Squid Agar (SQ)	DMSO
Cooked Meat Agar	
Tryptone-Yeast Extract Agar (TYE)	
FA IV	
NZABT	

TABLE 4. Nutrient Formulations Tested (See Appendix)

media for luminescent bacteria. Dimethylsulfoxide (DMSO), a research compound known to aid in transport of chemicals through membranes, was also tested. Medium formulations with DMSO did not increase sensitivity to narcotics.

Results of these tests have shown that two formulations, Lum and Squid agars, in conjunction with these same two broth formulations produce consistently better biosensors for narcotics than any other formulation. Two additional formulations (NZABT and FA IV) were inconsistent, but on occasion yielded biosensors with sensitivity exceeding that of either the Lum or Squid agar formulations.

In most cases, the inoculum source for growth medium evaluations was either Lum or Squid broth. Experience has shown these two formulations to be superior to others for culture maintenance. It should be mentioned, however, that the best biosensors with strain C-50 (and others) result from preparation of a Squid agar biosensor plate with Lum broth inoculum. This effect has been verified, with C-50 and other strains and is apparently related to enzyme induction (the stimulation of metabolic pathways due to presence of certain components of the growth medium) on transfer to the solid medium. The Lum broth to Squid agar inoculation is apparently more efficient in stimulating the metabolic pathways involved in narcotics sensitivity than either Lum to Lum or Squid to Squid transfers.

SENSOR SELECTION AND PRESERVATION

The selection of the optimal biosensor strains developed during the course of this program, and preparation of these strains for use in the feasibility demonstrations required several additional tasks. These included an analysis of all available test data, sensitivity and discrimination tests utilizing a modified screening procedure, and a study of suitable preservation techniques for the biosensor.

The method of testing resulted in utilization of progressively fewer strains for progressively more detailed experiments. This was the result of continual data analysis during the course of the program. Strains incapable of detecting narcotics in any form, or incapable of providing consistent signals, or strains incapable of discriminating controls from test materials were not studied further. As mentioned earlier in the text, all strains were tested at a minimum of two ages on at least two different liquid culture media. Elimination of strains from further study, based on the above criteria, resulted in selection of twelve strains possessing one or more desirable characteristics, but all showing sensitivity and discrimination in narcotics detection. Analysis of data from the strain selection program resulted in selection of four of these strains as superior. The four selected were the unanimous, independent choices of three staff microbiologists, each with experience in this program.

Additional studies were conducted with these four strains, utilizing modified and more detailed methods of screening. These included detection of field or simulated field-type narcotics packages, an analysis of

automatic sampling methods, and testing with several RPC Corporation prototype detectors.

The modified screening methods used, involved the use of a series of identical sample containers, each containing either a different form of narcotic, a narcotic simulant or interferent, or a control.

Narcotics utilized included: sealed and unsealed bulk heroin (85-87%) wrapped in plastic and newspaper; loose, 85-87% heroin; 250 glassine street bags of heroin; a marijuana brick; and marijuana cigarettes. The simulants, interferents, and controls (listed in Table 5) were tested both loose in the sample container and wrapped as the narcotic they simulate (using equivalent volumes). Testing with this series of sample containers utilized the laboratory test apparatus for comparisons between different strains or treatments, and an RPC Corporation prototype detector (Model 70-6) for analysis of a strains' ability to discriminate between narcotic and non-narcotic materials. Examples are shown in Figure 16.

Additionally, the prototype detectors (Models 70-5,6), which were to be used for the actual demonstration, were utilized for testing without the necessity of interpreting a strip chart recording as evidence of detections. This is possible because of the incorporation of a programmable alarm circuit. This circuit provides selectable audible and visual indicators of desired alarm conditions. Once programmed for the desired signal or detection condition, only that condition will yield an alarm. Thus, inexperienced personnel with some training in sampling technique can interpret detections without recourse to analysis

Heroin	Marijuana	Controls
Flour Lactose Sucrose Powdered Milk Camphor	Oregano Alfalfa Tea Cigarettes Cigarette Tobacco	Newspaper Empty Container Empty Glassine Bags with tape.

TABLE 5. Controls, Narcotic Simulants and Interferents Used in Screening.

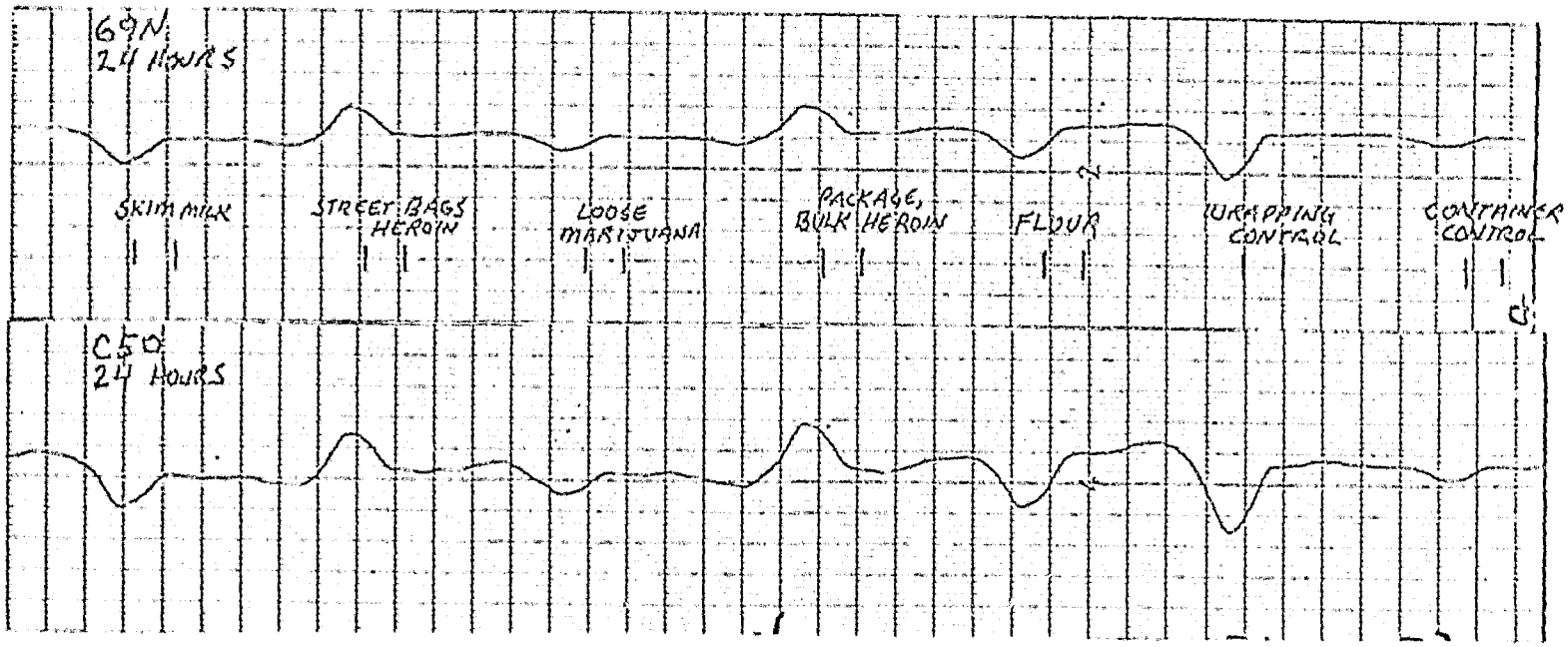


Figure 16. Sample Strip Charts Showing Ability of the Biosensors to Discriminate Between Narcotic and Non-Narcotic Substances. Chart speed was 1 mm./sec. and recorder sensitivity was 500 mv/div. Both biosensors were prepared on Squid agar from Squid broth. Sample times are indicated by the vertical marks between the pen traces. (See also Figure 18).

of strip chart waveforms. Strip chart recordings were made to provide a permanent record of testing.

Testing with these methods showed that the most consistent, reliable detections of heroin (in any form) were made with C-50, a new isolate from domestically obtained Squid (see also Mutagenesis Sections for a discussion of several mutants of C-50). A second choice, better than other strains but not as good as C-50, was 69N, a Japanese isolate. However, neither culture was useful for detection of marijuana. Both strains were shown to be capable of yielding signals with any form of heroin, while yielding a signal of opposite polarity or no signals at all with the interferents or controls. Under some circumstances, (rapid relative humidity fluctuations), the controls yielded a very diminished signal of the same polarity as the heroin signal. However, this condition did not yield false positive alarms, even with the relatively simple alarm circuits integrated in the Model 70-6 prototype detector.

Automatic sampling methods (see Engineering Section) were also tested both with the laboratory test apparatus and with the prototype detector. (The latter has built-in pulse amplifier-controller circuits). The reference probe may sample environmental (laboratory) air, or the air from a control container. In all cases, the instrument automatically alternates between the reference and test sources. In most cases, strip chart records were made, but alarm circuits were utilized as evidence of detections. The tests showed that this method, referencing against flour or skim milk, is potentially useful in eliminating the small amplitude signals obtained under conditions of changing relative

humidity. Use of this method is essentially a differential sampling method in that no signal is generated, even with narcotics, if both probes sample the same source; only signal differences between the sources are processed, thus eliminating relative humidity problems by constant reference to the source yielding the potential problem signal. As an additional benefit of using this method, no human timing decision or manipulation, other than probe to source insertion, is needed. Figure 17 shows a sample of results obtained with this method.

Analysis of signals obtained with automatic reference sampling provided additional evidence that C-50 and 69N were the optimal strains for heroin detection, based on all criteria. To test the ability of these strains to provide reliable detections over long time periods, studies were conducted to analyze preservation techniques and strain stability.

Techniques studied included lyophilization (freeze-drying), the method of choice for most long term storage of microbial cultures. In this method, a liquid microbial cell suspension is frozen at -70°C, and dried and sealed under vacuum. Storage of the resultant pellet, contained in a vacuum sealed glass tube, requires no refrigeration or special handling. Cultures maintained in the lyophilized state have been shown to remain viable and stable for physiological characteristics (such as sensitivity to narcotics) for periods exceeding 10 years. Reactivation of the pellet may make use of an intermediate liquid culture medium stage, with subsequent inoculation onto a solid medium, or the pellet may be directly applied to the solid medium. Use of the

Figure 17. Sample Strip Chart Recordings of Automatic Sampling Techniques. In all cases, the reference gas was laboratory air, the chart speed was 1 cm./sec., and the recorder sensitivity was 500 mv/div. The biosensor utilized was a 24 hour C-50, prepared on Squid agar from ... broth. Narcotics are shown in the left column, and controls and ...ants in the right column. Sample time was 5 seconds and the repetition rate was 25 seconds. The vertical marks below the pen traces on each chart indicate sample times for one pulse, and phasing for the sensor. The flour, skim milk, and lactose controls contained the individual controls wrapped to simulate the bulk heroin packages. The street bag control contains empty glassine bags, sealed with plastic tape to simulate the actual street bags.

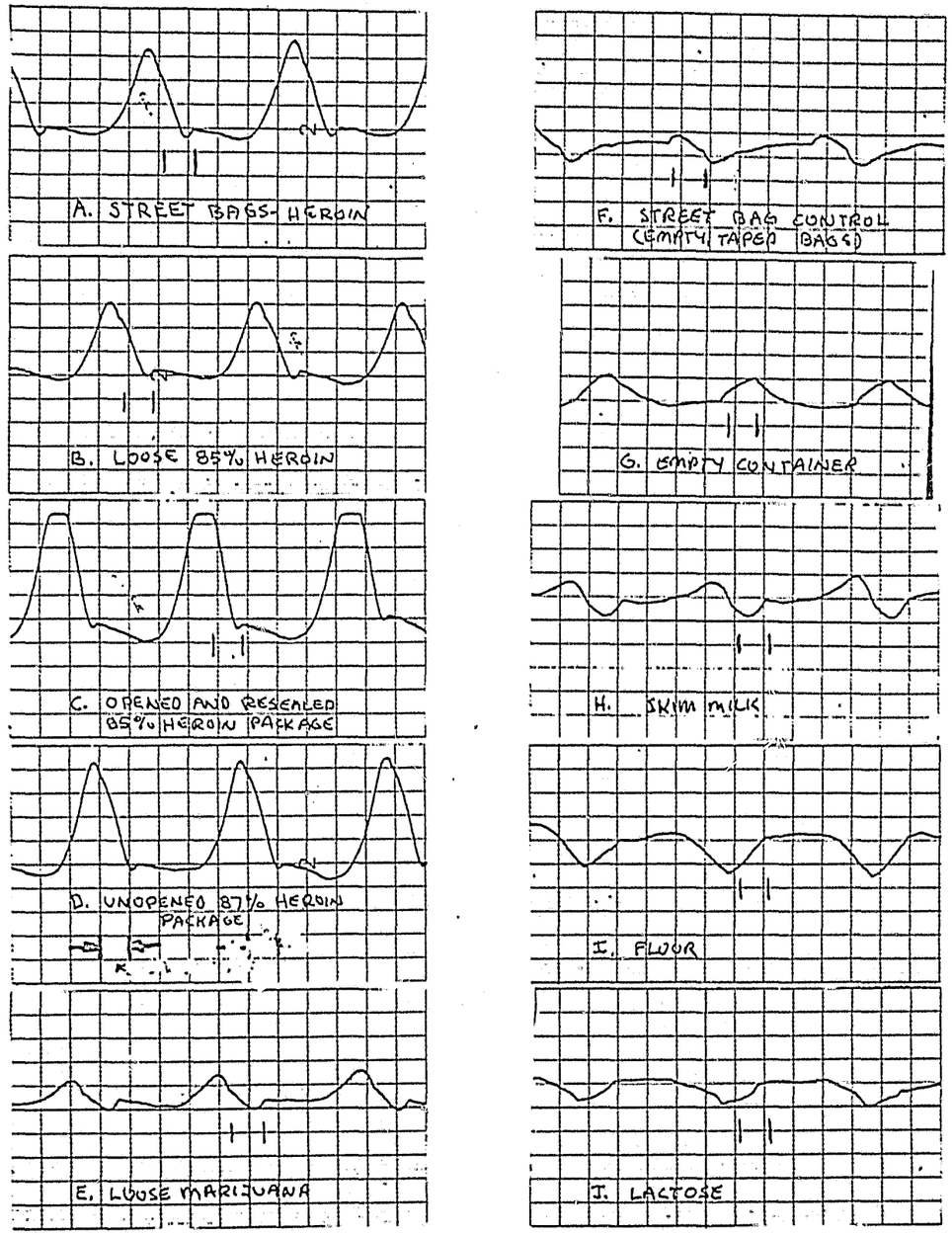


Figure 17. Sample Strip Chart Recordings of Automatic Sampling Techniques.

former method, though slightly more cumbersome and time consuming, provides shorter incubation times to attain useful-age biosensors. However, both methods proved equivalent in yielding useful biosensors.

Additional preservation methods included: use of refrigerated, solid medium cultures, with and without mineral oil overlay, with and without the intermediate liquid medium stage; non-refrigerated, non-overlaid solid medium; and refrigerated and non-refrigerated liquid medium storage.

All methods tested proved capable of short term (2 week-6 month) storage of the biosensor strains. The limiting factor was shown to be the relatively short useful life (without inoculation to fresh medium) of the intermediate liquid medium culture. This liquid culture, whether used as the primary storage method (transferred at 2 week intervals), or as an intermediate stage for other methods was limited to a two-week life span for use in preparation of biosensors. However, during this period the liquid culture was capable of providing inoculum material for the preparation of reliable biosensors. Figure 18 shows a representative sample of results.

C-50 was found to be stable in physiological characteristics when stored under a variety of conditions. However, both test data and a priori reasoning suggest that lyophilization is the method of choice for long term storage.

Figure 18. Sample Strip Chart Recordings Showing a Comparison of Lyophilized and Broth Culture Maintained Biosensors. Charts show the response of 24 hour, C-50 biosensors prepared from: A, the broth used for preparation of biosensors for the New York demonstrations; B, a 12 day broth culture maintained at RPC Corporation laboratories without refrigeration; and C, a biosensor prepared from a lyophilized pellet inoculated into Lum broth. Chart speed was 1 mm./sec. and recorder sensitivity was 500 mv/div. All sensors were prepared on Squid agar from Lum broth. Sample intervals are shown below Chart C.

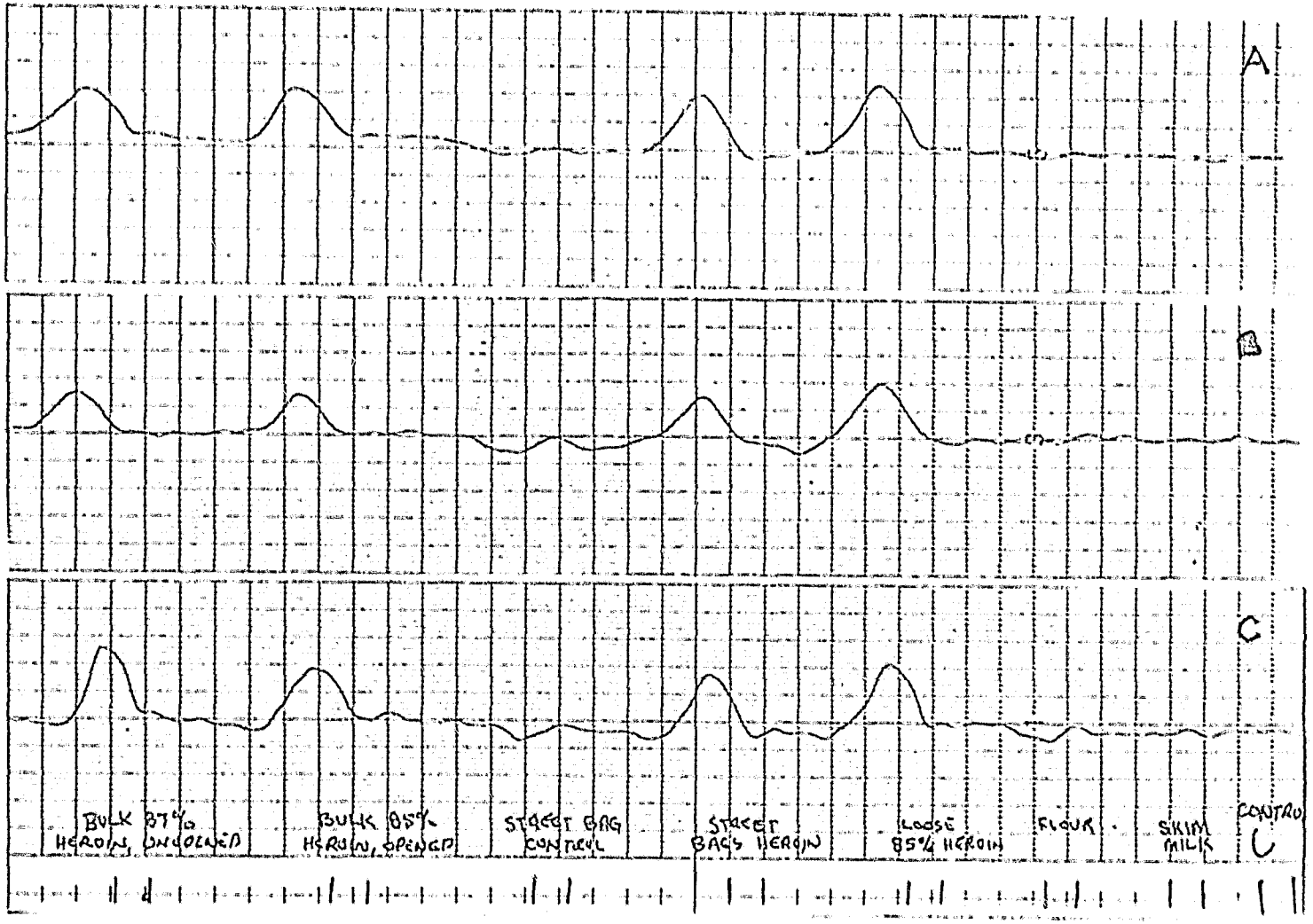


Figure 18. Sample Strip Chart Recordings Showing a Comparison of Lyophilized and Broth Culture Maintained Biosensors.

FEASIBILITY DEMONSTRATIONS

As a contractual requirement, feasibility demonstrations were planned. These were to include a demonstration of the ability of the bioluminescence concept to rapidly detect various forms of narcotics, and provide an indication of detection (alarm) without interpretation of strip chart recordings. Additionally, narcotic control substances and simulants were to be sampled to demonstrate the specificity of the system. These methods were successfully utilized at an earlier stage in the program in a demonstration at the RPC laboratories for members of the New York Police Department.

Based on all available data, strain C-50 was selected as the biosensor for use in the feasibility demonstration, with strain 69N selected as an alternate. Actual preparation for the demonstration commenced one week earlier than the actual demonstration, to allow time for testing under the actual field conditions. Although both selected strains have been shown to be quite stable to all factors, including incubation environment (for biosensor preparation and testing) verification of this in New York was desirable.

These tests, utilizing the Model 70-6 detector and the methods described above, were conducted in the Bomb Squad laboratory and in the Auditorium of the New York Police Academy. Biosensor preparation and culture storage was performed in the test personnel's hotel room. During this one week period, all tests conducted yielded results equivalent to those obtained at RPC Corporation. These tests were witnessed and verified in part, by New York Police Department personnel.

In spite of all preparation, technical difficulties with the biosensor did not permit a demonstration of narcotics detection on the scheduled date (December 17, 1970) at the Police Academy. All evidence indicates that the combination of high temperature and low relative humidity on the day of the test, and higher than normal biosensor incubation temperature at the hotel on the day preceeding the test resulted in biosensors incapable of discriminating narcotics from non-narcotic material. This is indicated by the fact that the biosensors prepared from the same source, but with proper incubation temperature were used in a successful demonstration on December 18, 1970 in the Mayor's Chambers, City Hall. Furthermore, biosensors prepared from the same source after returning to the RPC Corporation laboratories proved capable of yielding detections of heroin, and discriminating narcotic and non-narcotic materials. (see Figure 18).

APPENDIX

FORMULAE

Luminous Agar (Lum)

Phytone (BBL)	8.0 g
Glycerol	5.0 ml
NaCl	30.0 g
$CaCO_3$	3.0 g
Agar (BBL)	23.0 g
H_2O (De-ionized)	1.0 L

Luminous Broth (Lum Broth)

Nutrient Broth (BBL)	8.0 g
Glycerol	5.0 ml
NaCl	30.0 g
$CaCO_3$	3.0 g
H_2O (De-ionized)	1.0 L

Veal Infusion Agar (VIA)

Veal Infusion (BBL)	40.0 g
NaCl	25.0 g
Glycerol	5.0 ml
Agar (BBL)	8.0 g
H_2O (De-ionized)	1.0 L

Thiotone-Yeast Extract Agar (TYE)

Thiotone (BBL)	5.0 g
Yeast Extract (BBL)	5.0 g
Glycerol	5.0 ml
$CaCO_3$	3.0 g
NaCl	30.0 g
Agar (BBL)	23.0 g
H_2O (De-ionized)	1.0 L

Squid Broth (SQ Broth)

Polypeptone (BBL)	2.5 g
Trypticase (BBL)	5.0 g
Yeast Extract (BBL)	0.5 g
NaCl	15 g
K_2HPO_4	1.25 g
Squid Extract	12.5 ml
Glycerol	2.5 ml
H_2O (De-ionized)	500 ml
(for Squid Agar, add Agar	10 g)

Lum Agar + Heroin (Lum + H)

Prepare Lum Agar and add 1% w/v Heroin aseptically after sterilization.

Lum Agar + Marijuana Extract (Lum + MJ)

Phytone (BBL)	4.0 g
Glycerol	2.5 ml
NaCl	15.0 g
$CaCO_3$	1.5 g
Agar (BBL)	11.5 g
H_2O (De-ionized)	150 ml
Marijuana Extract	100 ml

Marijuana Extract

Marijuana	25 g
H_2O (De-ionized)	500 ml

cold extract: allow to sit 24 hours, filter through Whatman #1 paper.
hot extract: boil to ½ volume, filter as above.

Luminous Agar with Caffeine (Lum-Caf)

Prepare standard Lum Agar and add:

1.0 ml of 5.0 g/L caffeine.

Luminous-Blood Agar (Lum-Blood)

Prepare standard Lum Agar and add:

6.25 ml/500 ml packed blood cells.

Cooked Meat Agar

Cooked Meat Phytone (BBL)	12.5 g
NaCl	7.5 g
Glycerol	1.25 ml
Agar (BBL)	5.75 g
H_2O (De-ionized)	250 ml

Dimethyl Sulfoxide Agar (DMSO)

Prepare standard Squid Agar and add:

DMSO (0.5, 1.0, or 1.5%)

NZABT

Prepare standard Lum Agar, substituting for Phytone:

N-Z-Amine Type BT (Sheffield) 8.0 g/L

FA IV

Prepare standard Lum Agar, substituting for Phytone:

Ferm Amine Type IV,
(Sheffield) 8.0 g/L

Chemical Mutagen Stock Solutions (Prepared in 3% Saline)

5-Bromouracil - 500 µg/ml, diluted 1:10 for use.

2-Aminopurine - 5000 µg/ml, diluted 1:10 for use.

Acridine Orange - 50 µg/ml, diluted 1:10 for use.

Nitrosoguanidine - add one crystal per 4.5 ml cell suspension.

GLOSSARY

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BACKGROUND - the normal gaseous environment.

BIOSENSOR - collectively, a mature colony of a microbial strain and its growth medium.

BIOSENSOR AGE - the time elapsed from inoculation of a biosensor.

BROTH - a liquid nutrient formulation used for the cultivation of microorganisms.

CHART SPEED - the time interval between points on a strip chart recording.

COLONY - a mass of microbial cells, visible to the naked eye, and of common origin; commonly a discrete mass on the surface of solid culture medium.

CULTURE - a group of microbial cells of the same strain.

DRUG DEPENDENCE - the condition where a physiological trait is dependent on the presence of a drug in the culture medium.

EFFLUENT - vapors or gases from a given source.

FLOWMETER - a device for measuring and adjusting flow.

GAIN - degree of amplification.

GAS SAMPLING SYSTEM - collectively, all components of a detector which come in contact with the sampled gas including sample probe, exposure chamber, flowmeter, pump, and connecting tubing.

INCUBATION - storage of microorganisms at the optimum temperature and humidity for reproduction and growth.

INDUCTION - in reference to enzyme production, the process of selectively stimulating production or activation of an enzyme.

INOCULATE (INOCULATION) - the process of initiating microbial growth in a suitable medium by transferring a small quantity of live cells.

INOCULATION GEOMETRY - the gross configuration of inoculated area on the biosensor plate or petri dish.

INOCULUM - a small quantity of living culture material or cells used for inoculation.

INOCULUM AGE - the age of the material, or inoculum, used for preparation of a biosensor.

INTENSITY - level of light emission.

ISOLATION - the process of obtaining microorganisms from a source in nature.

LYOPHILIZATION (FREEZE-DRYING) - the process of preserving microorganisms by drying frozen culture material under vacuum.

MUTAGEN - a chemical or physical agent capable of causing mutations.

MUTAGENESIS - the process of inducing mutations.

MUTANT - an organism or culture which, due to spontaneous or induced mutation, differs from the parent culture in one or more heritable characteristics; the result of mutagenesis.

NOISE - normal small amplitude, random fluctuations in a signal, generated by the biosensor itself or the electronic circuits.

NUTRIENT AGAR - a solid, gelatinous substrate, containing appropriate nutrients used for the culture of microorganisms.

PELLET - lyophilized culture material.

PETRI DISH - a standard culture container filled with nutrient agar used for growth of microorganisms.

PHOTOCHEMICAL SENSOR UNIT (PSU) - refers collectively to the exposure chamber, sample inlet and exhaust ports, biosensor holder, photocell, and (in some cases) integral pre-amplifier.

PHOTOSENSOR - a transducer for converting light intensity signals into electrical signals.

QUIET BACKGROUND - a background with low noise level; the condition where the normal gaseous environment does not result in excessive noise levels.

RATE - change in signal value in time.

SCREENING - the process of comparing biosensor response to a given parameter.

SENSITIVITY - the characteristic of responding, in a definable manner, on exposure to an effluent.

SHOOTING GALLERY - a location favored by addicts for injection of heroin.

SIGNAL - the characteristic electronic information resulting from exposure of a biosensor to an effluent.

SIGNAL CONDITIONER - an electronic circuit or circuits used to process, convert, and amplify signals.

SPECIFICITY - refers to the ability of a biosensor, or detector, to react to certain effluents while reacting differently, or not at all, to other effluents, related or unrelated.

STRAIN - a laboratory culture of a microbial species, with distinct physiological or morphological characteristics.

STRAIN SELECTION - the systematic application of various mutagenic and/or physiological techniques to a strain of microorganisms, aimed at developing or enhancing pre-existing characteristics such as sensitivity to narcotics.

TRANSDUCER - a device for conversion of one form of energy to another.

END