

Biosensor Standards Requirements

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Since biosensor applications are springing up in many places, it should be the wish of all biosensor manufacturers, their customers, as well as those in academia, that the same language and requirements are used to describe similar products. In order to find answers to the question as to whether the time is right to consider developing standards for biosensors, and if so, what the priority areas might be, we have conducted a survey of biosensor users and/or manufacturers. It is the purpose of this article to report our survey results.

INTRODUCTION

A biosensor is a device that incorporates a biological sensing element in close proximity or integrated with the signal transducer (Figure 1), to give a reagentless sensing system specific to a target analyte (Hall, 1991). Usually a biosensor can be used by nonspecialist operators, either continuously on-line or discretely as a throw away device. Transducers are the physical components of the sensor that respond to the products of the biosensing process and outputs the response in a

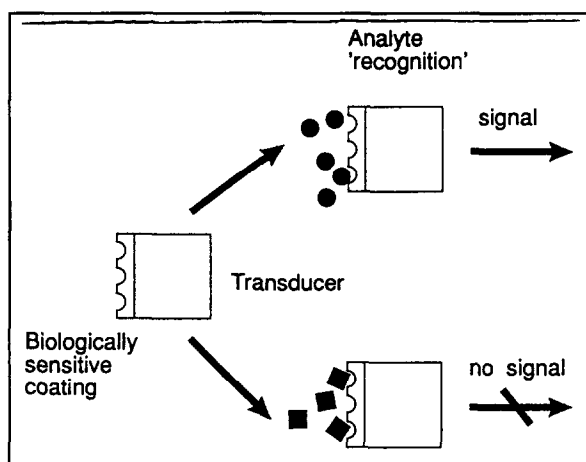


Figure 1: The biosensor: surface modified transducer which is reactive towards a specific analytic.

form which can be amplified, stored, or displayed. Several types of transducers, including ion-sensitive electrodes, ion-sensitive field effect transistors, amperometric electrodes, potentiometric devices, calorimetric devices, optical transducers and piezoelectric crystals, have been used in biosensors.

All these transducers can be grouped into four main classes, electrochemical, mass, optical and thermal (Janata, 1989).

Electrochemical detectors are the oldest and most popular. Their mode of operation includes measuring voltage (potentiometric), current (amperometric), conductivity (conductimetric).

Examples of mass detectors include piezoelectric crystals and surface acoustic wave devices.

Most optical detection processes rely on the absorption of light, where there is a quantitative relationship between the concentration of the absorber and the fraction of light absorbed.

Thermal detectors use the heat generated by a specific reaction as the source of analytical information. Example of thermal detectors include thermistors and pyroelectric devices.

A recent study by Frost & Sullivan, "The Biosensor Market in the US", projected demand for biosensors to reach \$69 million by the year 1993 (Keeler, 1991). With biosensor applications

Table 1: Biosensor Applications

General Area	Example
Healthcare	blood glucose monitoring at home
Process monitoring	control of fermentation reactors
Quality control	fish freshness
Environmental	BOD of industrial effluent
Military	biological and chemical warfare

springing up in numerous fields (Table 1), it should be the wish of all biosensor manufacturers, their customers, as well as those in academia that the same language and requirements are used to describe similar products. In order to find answers to the question whether the time is right to consider developing standards for biosensors, and if so, what are the priority areas, we conducted a

survey of biosensor users and/or manufacturers and the purpose of this paper is to report our survey results.

It should be pointed out that the survey was done on behalf of the American Society for Testing and Materials (ASTM), Biotechnology Subcommittee E48.03—Unit Processes and their Control. ASTM is a non-profit organization devoted to the development of voluntary and consensus standards for materials, products, systems, services and the promotion of related knowledge.

One hundred and forty-eight company names were obtained from the 1992 *Genetic Engineering News* guide to biotechnology companies. All the companies included in the survey listed Process Monitoring and Control among their technologies of interest. Out of the 148 copies sent the questionnaire, 26 (17%) were returned and 22 qualified for use in data analysis. The questionnaire was prefaced by a guide note which defined the terms biosensor and transducer (see Appendix A).

Table 2: Characteristics of respondents.

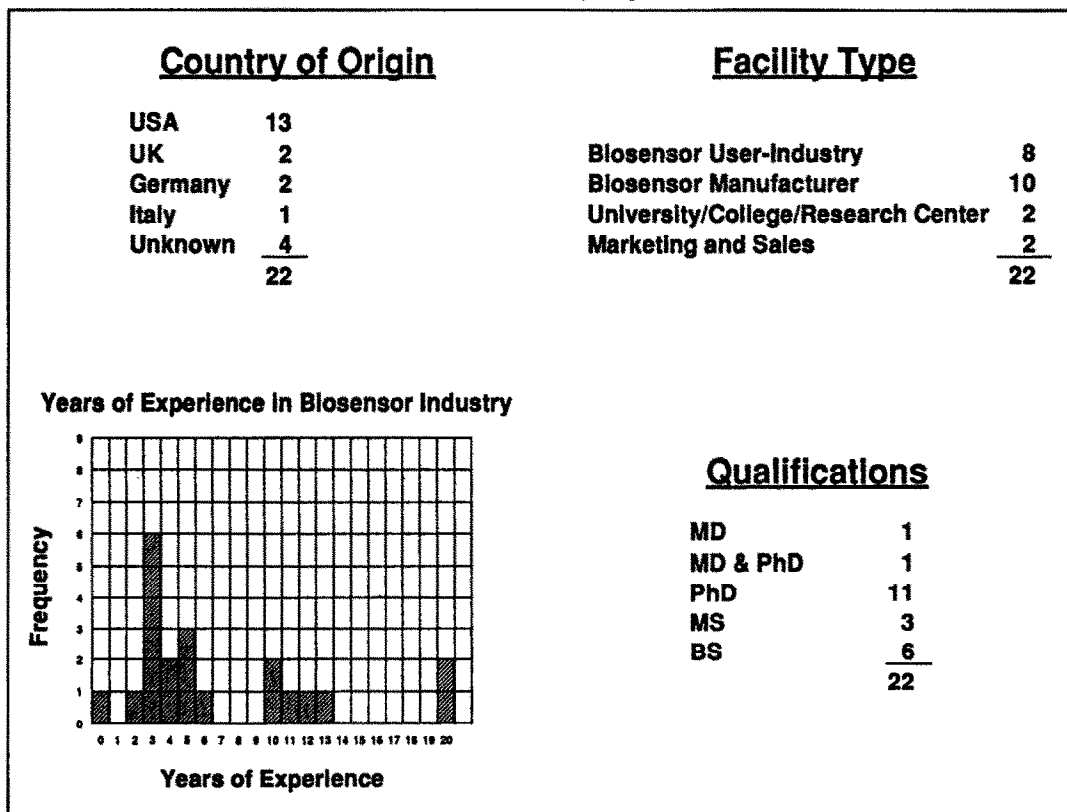


Table 3: Characteristics of respondents.

Most Familiar Technology		Most Familiar Applications	
Technology	Times Cited	Application	Times Cited
Antibody/Antigen/Receptor	12	Healthcare	13
Enzyme	12	Process monitoring	13
Microbial	7	Quality control	9
Other	4	Environmental	8
		Military	4
		Food Analysis	2
		Illicit Substance	1
Most Familiar Transducer Technology		Product Commercial Status	
Technology	Times Cited	Status	Times Cited
Optical	15	Commercially available	10
Electrochemical	14	6 months to launching	3
Mass	3	1-5 years to launching	6
Thermal	1		

As shown in Table 2, the majority of respondents (59%) were from the USA, 50% held PhD degrees and 50% worked at biosensor manufacturing facilities. Most respondents had 2-6 years of experience in the biosensor industry, a reflection of the evolving nature of the industry.

The most familiar biosensor recognition and transducing technologies to most respondents were antibody/receptor and optical types, respectively (Table 3).

It was interesting to find that healthcare and process monitoring applications were each cited 13 times as one of the most familiar applications, given that the companies in the database were involved in process monitoring and control. This may suggest that most of facilities surveyed are manufacturing or developing biosensor with applications in both fields. As shown, ten commercially available products were cited.

IS THERE A NEED TO DEVELOP BIOSENSOR STANDARDS?

The tally of the responses to this question are summarized in Table 4.

Table 4: The need to develop standards based on recognition technology.

Technology	Average Score ± 95% Confidence error
Enzyme	3.93 ± 0.42
Microbial	3.82 ± 0.45
Antibody/Antigen/Receptor	3.55 ± 0.48

Enzyme technology was the leading candidate. This was not surprising because enzyme-based technology is the oldest with its beginnings in the mid-1950s, when Lerand C. Clark, Jr., of the Children’s Hospital Research Foundation in Cincinnati, OH, USA, invented a dissolved oxygen electrode for patients undergoing surgery. In 1962 the technology was extended to sensing blood glucose levels by coating the oxygen electrode with a layer of gel containing glucose oxidase, followed by a semipermeable dialysis membrane to contain the

enzyme while allowing glucose to diffuse through. The membrane also prevented entry of undesirables.

The world's most successful biosensor to date, the ExacTech glucose sensor, manufactured by MediSense (Boston, MA, USA) is based on technology similar to that of Clark's device but with some improvements. Oxidase systems with amperometric detectors are expected in the near future to continue to dominate the technology of commercial biosensors.

There are considerable research efforts both in industry and academia for novel oxidases. Examples include glutamate oxidase (Yamasa Shoyo, Japan), NADH oxidase (Toyo Jozo, Japan), and phenylalanine oxidase (Gamati and Luong, 1991).

Other efforts are focussed on improving immobilization technologies, enzyme activity and long term stability (Luong et al., 1991).

In a recent literature survey, 75% of commercial biosensors were enzyme-based (Kisaalita, 1991).

The low score for antibody/antigen/receptor recognition technology was not surprising. Despite the early enthusiasm for the detection of the binding of immunoreagents directly by an electrode, to date no true commercial biosensor has been introduced based on antigen-antibody interactions. Many promising early results have now been attributed to experimental artifacts (Foulds et al., 1990). Also, high binding constants involved in immunochemical reactions that result in very poor reversability have hampered

competitive binding biosensor development efforts. Instead, therefore, we have seen the emergence of immunoassay devices. For example, Westinghouse will be launching the "illicit substance detector" for cocaine in the near future, and Pharmacia is marketing a surface plasmon resonance mass detector, "BIAcore", for protein-protein interactions. The BIAcore device is designed for research applications where the environment is very well controlled.

The high score for microbial-based technology was surprising. Although several microbial biosensors have so far been developed for glucose, acetic acid, ethanol, methanol, ammonia, nystatin and trimethylamine (Karube, 1986; Gamati et al., 1991; Luong, et. al., 1991), there are major drawbacks from a commercial standpoint. These comprise poor selectivity and sluggish response times. Therefore this category of biosensors is expected to remain in the research laboratories where samples are usually very well defined.

IS THERE A NEED TO DEVELOP INDUSTRY-WIDE BIOSENSOR PERFORMANCE CHARACTERISTICS STANDARDS?

Among the terms shown in Table 5 with their possible interpretation, published by Janata (1989), the top-ranked characteristics were reproducibility, selectivity, stability and sensitivity (Table 5).

Table 5: Terminology of Biosensor Performance Characteristics.

Term	Meaning
Sensitivity	The slope of the response curve expressed as output per unit concentration.
Selectivity	The ability of the device to measure one chemical component in presence of others.
Dynamic Range	The range of concentrations in which the sensitivity is greater than zero.
Response Time	The time at which the output reaches 63% (1/e) of its final value in response to a step change in concentration.
Reproducibility	Accuracy, usually expressed as variance, standard deviation or coefficient of variation.
Detection Limit	The concentration at which the mean value of the output is equal to two standard deviations.
Life Time	The usable period of the biosensor, either as "shelf" or "in-use".
Stability	The percent change of the baseline and/or sensitivity in time.

Table 6: The need for industry-wide standards for biosensor performance characteristics.

Performance characteristics	Average Score ± 95% Confidence error
Reproducibility	4.76 ± 0.23
Selectivity	4.59 ± 0.35
Stability	4.41 ± 0.48
Sensitivity	4.14 ± 0.52
Dynamic Range	3.86 ± 0.54
Life Time	3.73 ± 0.62
Response Time	3.36 ± 0.62
Deflection Limit	(N/A)

Other — Sanitary protection
— Truth in advertising

It was interesting to find that the 95% confidence error increased with decreasing score order, suggesting that there was consistent agreement among respondents as to the relative significance of the performance characteristics. (Also, detection limit was misspelled on the questionnaire as detection list!) The average score of 3.1 (not sure) for detection list further confirmed the accuracy of these results.

Table 7: Materials and/or components for which industry-wide standards would be useful.

Recognition Technology	Material and/or component
enzyme / microbial	enzyme, mediators electrode materials m/w profiles/cut off (membranes)
MoAb / Ag / Receptors	fluorophores light sources / detectors
Common	calibrators polymer supports flow immobilisation chemistries transducers autoclavable connectors

Another characteristic brought to our attention by one of the respondents was sanitary protection. One respondent mentioned the need for truth in advertising, which further underscores the need of standards. Suggestions from respondents for materials/or components for which industry-wide standards would be useful are summarized in Table 7 and the most frequently mentioned item was enzymes.

SUMMING UP

The results of this survey not only confirmed the need to develop standards for biosensors, but also clearly identified the priority areas. As a first step, ASTM Subcommittee E48.03 (Unit Processes and their Control) is in the process of drafting standard test methods for several biosensor performance characteristics.

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development of voluntary and consensus standards for materials, products, systems, service, and the promotion of related knowledge. Answers to all the questions will tell us whether the time is right to consider developing standards for biosensors and if so, what the priority areas are. We highly value your opinions and ideas.

Sincerely

William S. Kisaalita, Ph.D.
Chairman ASTM Biotechnology
Subcommittee 48.03
(Unit Processes and their Control)

APPENDIX A

Dear Biosensor User and/or Manufacturer:

We are taking a survey of a selected number of professionals working in the biosensor industry. We are particularly seeking their personal opinions (and not their company position or propriety information) regarding the issue of standards. We understand you or your company have an interest in biosensors. We would be most grateful if you or someone else in your company would complete this survey and return it to us in the enclosed postage-paid envelope.

The questionnaire was designed by the American Society for Testing and Materials (ASTM), Biotechnology Subcommittee 48.03. ASTM is a non-profit organization devoted to the

QUESTIONNAIRE ON BIOSENSOR STANDARDS

A biosensor is a device that incorporates a biological sensing element in close proximity or integrated with the signal transducer to give a reagentless sensing system specific to a target analyte. Usually a biosensor can be used by non-specialist operators, either continuously on-line or discretely as a throw away device. Transducers are the physical components of the sensor that respond to the products of the biosensing process.

On the scale below (Items 1 to 4) make a check to indicate your agreement with these statements.

- 1. There is a need to develop standards for **antibody/antigen/receptor-based biosensors.**

strongly disagree		not sure		strongly agree
1	2	3		4
5				

- 2. There is a need to develop standards for **enzyme-based biosensors.**

strongly disagree		not sure		strongly agree
1	2	3		4
5				

- 3. There is a need to develop standards for **microbial-based biosensors.**

strongly disagree		not sure		strongly agree
1	2	3		4
5				

- 4. There is a need to develop standards for other biosensor types (specify).....

strongly disagree		not sure		strongly agree
1	2	3		4
5				

- 5. Check on the scale to indicate your views regarding usefulness of industry-wide standards for each biosensor performance characteristic.

use-	not	use-
less	sure	full
1	3	5

- a. Sensitivity
- b. Selectivity
- c. Dynamic range
- d. Response time
- e. Reproducibility
- f. Detection Limit
- g. Life time
- h. Stability
- i. Other (specify)

- 6. List materials and/or components utilized in biosensors for which industry-wide standards would be useful.

- 7. Biosensor technology you are most familiar with (circle all that apply).

- a. Antibody/Antigen/Receptor
- b. Enzyme
- c. Microbial
- d. Other (specify).

- 8. Biosensor applications you are most familiar with (circle all that apply).

- a. Healthcare
- b. Process monitoring
- c. Quality control
- d. Military
- e. Environmental
- f. Other (specify).

- 9. Transducer technology you are most familiar with (circle all that apply).

- a. Electrochemical
- b. Optical
- c. Mass
- d. Thermal
- e. Other (specify).

over

- 10. Your facility type (circle one)
 - a. Industry-Biosensor user
 - b. Industry-Biosensor manufacturer
 - c. University/College/Research Center
 - d. Hospital/Medical Center
 - e. Government Lab
 - f. Other (specify)

- 11. Commercial status of your current product (circle one).
 - a. Commercially available
 - b. To be launched within 6 months
 - c. To be launched within 1-5 years
 - d. Other (specify).

- 12. Indicate years of experience in biosensor industry.

- 13. Your qualifications (circle one or more).
 - a. MD b. PhD c. MS d. BS

 - e. Other (specify).

- 14. Indicate your primary title.

- 15. Any additional comments?

- 16. Would you like to receive the results of this survey? If yes, please complete items 16, 17 and 18 below. Your identity will be kept confidential.

- 16. Name

- 17. Address

- 18. In case we have follow-up questions, can we contact you again? (circle one).
 - a. No
 - b. Yes

If YES, what is your phone number?