A Survey of Bioassays in the Biopharmaceutical Industry

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Bioassays represent a major investment in development, production, and licensing of most biopharmaceuticals. They present unique issues compared with physicochemical analytical techniques in their susceptibility to variables that may be difficult to identify or control.

Biological assays, especially potency assays needed to release product, are often poorly understood and greatly feared. Companies therefore tend to initiate their development too late and without a clear idea of how each assay should be used and formatted. Compounding these problems is the current lack of regulatory guidance.

As a result, specific concerns associated with bioassays are encountered widely throughout the industry. It is to the advantage of all parties concerned — industry, regulators, and consumers — to identify and address some common issues and problems.

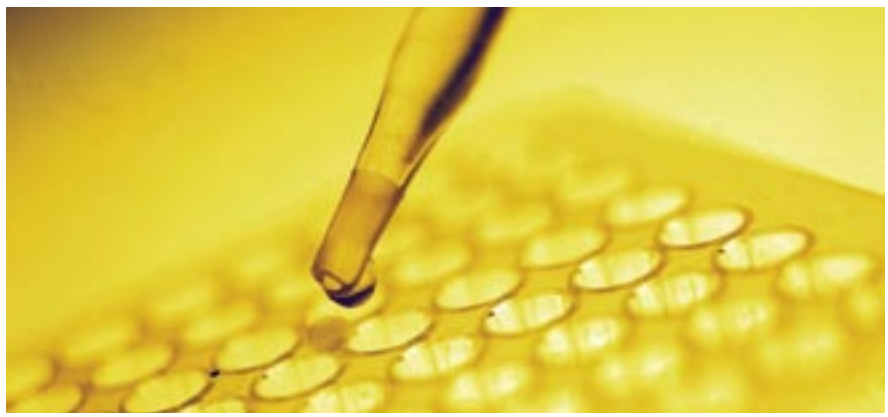
PRODUCT FOCUS: MOST BIOPHARMACEUTICALS, INCLUDING VACCINES AND DIAGNOSTICS

PROCESS FOCUS: ASSAY DEVELOPMENT

WHO SHOULD READ: PRODUCT DEVELOPMENT, QA/QC, REGULATORY AFFAIRS, PROCESS DEVELOPMENT, AND FORMULATION

KEYWORDS: BINDING ASSAYS, BIOASSAYS, IMMUNOASSAYS, FUNCTIONAL ASSAYS, AND OTHER ASSAY TYPES

LEVEL: BASIC



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At the 2004 IBC European Biological Assays conference (Berlin, 19–20 October), we surveyed the delegates' use of bioassays in their companies. The answers indicated several issues of common concern. Based on those 2004 survey results, and given the interest shown by the industry in them, a more detailed survey was designed and then circulated among the delegates at the 2005 IBC European Biological Assays conference (Basel, 18–19 October). The results of this second survey are published here to provide data on current bioassay use and subjects of concern. These are intended

Question 1: The companies (40 responses to 1A and 1B; 37 to 1C, with two companies reporting more than one primary location)

The Company		Primary	Other
A) Location	Europe	31	5
	United States	9	16
	Japan	2	8
	Asia	1	6
	Elsewhere	1 (Mexico)	2 (Global, South America)
B) Size		All Companies	European Primary Location
	< 50 Employees	6	6
	50–249	9	7
	≥ 250	25	18
C) Business		All Companies	European Primary Location
	Early development	28	23
	Production	25	18
	Contract testing	5	5

for use by industry, regulators, funding bodies, and other interested parties.

Methods

Our survey comprised 13 questions concerning various aspects of bioassay use with company and product details. Conference delegates were asked to complete this survey anonymously. The conference was an appropriate forum because its delegates were expected to have an interest in and knowledge of bioassay use. This international meeting was the fourth of a series held in Europe; representatives from several countries participated, although European companies were represented particularly highly.

At the meeting, 99 delegates representing 79 organizations in 17 countries were registered. Delegates were asked that if colleagues from the same organization were present, they were to collaborate and submit only one form per organization. Delegates from organizations for which the survey questions were inappropriate were asked to return a form indicating why.

Question 2: Product type; number of companies with products of each type and each stage of development (40 responses)

	Therapeutic						
Product Type	Prelinical P1, P2a, P2b	Phase 3	To Market	Ancillary to Therapeutic	Diagnostic	Reagent	Other
Biological (nonbiotech)	11	7	8	6	3	1	2 vaccine 1 batch control
Biotech	28	15	15	6	3	1	1 vaccine 1 other
Non-biological	8	5	9	1			1 (lab supplies)

Question 3A: Importance assigned to the bioassay (40 responses)

Assigned by . . .	Very High	High	Medium	Low	Very Low
Management	3	11	19	3	1
Regulatory	6	18	7	2	1
QA	11	16	5	2	1

Question 3B: Resources assigned to the bioassay (36 responses)

	Yes			No		
Adequate?	15			21		
Company size	<50	50–249	≥250	<50	50–249	≥250
	2	4	9	4	5	12

Question 4: Bioassays — what types and when are they developed and used? (39 responses)

									Companies Reporting								
									Stage of Product Development When Bioassay Developed				Stage of Product Development When Bioassay Used				
	Assay Type	Companies Using	Total Established Assays	Preferred Format	Also Used	Used When No Alternative	GLP (Toxicity, Etc.)	GMP (Release Etc.)	Pre-clinical	P1	P2	P3	Pre-clinical	P1	P2	P3	Market
a	In vivo	17	47	1	2	5	5	13	11	2	1		7	4	3	4	4
b	1y cells	9	16	1		2	4	5	5	2		1	4	3	1	2	2
c	Cell lines	28	105	6	2	3	7	15	13	5	3	1	9	11	10	8	7
d1	Late stage readout (e.g., proliferation)	23	60	3	3	1	6	12	9	7	5	2	7	7	7	7	3
d2	Early readout (e.g., reporter gene)	12	33	5	1		1	5	4	3	2	1	4	2	2	1	
e	Binding to cells (e.g., FACS)	19	35	4	2	1	5	9	10	3	1		6	5	4	3	2
f	Binding without cells (e.g., Biacore)	14	21	2	3			4	5	1	1	1	3	2	2	1	1
g	Immunoassay (e.g., ELISA)	30	160	7	1	1	9	18	15	5	1	1	12	11	9	7	9
h	Enzyme activity	14	29	5	1		3	6	3	2	2	1	4	4	4	4	4
j	Other	1 phago-cytosis	1					1	1							1	

Question 5: Is there a consistent strategy of using certain bioassay types at a specific product development phase? (40 responses)

	Yes (12)			No (10)			Partial (14)			NA (4)		
Company size	<50	50–249	≥250	<50	50–249	≥250	<50	50–249	≥250	<50	50–249	≥250
	2	2	8	1	3	6	3	3	8		1	3

Question 6: The purpose for which the bioassay is used. Delegates were asked that only those using bioassays for GMP purposes complete this question[1]

Purpose of Bioassay	Companies Using Bioassays for This Purpose	Assay Types If Specified										
		a	b	c	d1	d2	"d"	e	f	g	h	j
Lot release	32	6	1	10	4	2	4	6	2	11	3	1
Stability testing	29	3	2	13	4	2	4	6	2	11	1	1
Formulation development	19	2		6	2	1	3	4	2	4	1	
Process development	22	3	1	8	3	1	2	5	1	7		
Characterization	22	2	1	7	5	2	2	6	4	8	1	
Comparability	24	3	2	8	6	2	2	4	3	5		
In-process control	13			1	1	1		2	1	7	1	
Qualifying reference standard	18	3		8	5	1	4	4	5	10	1	
Preclinical research	17	1	1	4	1	1	2	3	1	5	2	
Immunogenicity	12	1		3		1	2	2	1	3		
Identity	14	3		4	2	1		5	2	5		
Impurities	6			1				1		2	2	
Other	(No responses)											

[1] 22 companies reported use of bioassays for GMP purposes, specifying assay types used. The assay types are as defined in Question 4; 14 companies reported use without specifying assay types used; four companies did not report bioassays used for GMP; some assay types were reported only as "d," rather than "d1" or "d2," as shown in the table.

Question 8: Would delegates like to replace functional bioassays? Delegates were asked that only those using bioassays for GMP purposes complete this question (33 responses with data; three responses stating NA, one of which is described as a contract researcher)

Replace . . .	No	Some	All	With Binding Assays	With Physicochemical Assays
Existing functional assays	12	16	1	13	5
Functional assay for future products	5	17	4	19	10

A presentation was made explaining the intended purpose of each question and the way answers should be formulated. Particular requirements for answering specific questions and particular constraints in formatting the results are indicated against the relevant tables in our "Results" section. The survey questions were presented in the same sequence as reported here. The tabulated results for each question are formatted similarly to that of the question, although some adaptation proved necessary.

Results and Discussion

Forty-one survey forms were returned, one of which was from a regulator who commented that the questions were inappropriate for his organization. The remaining 40 forms were returned by representatives of biopharmaceutical companies and are reported here. On certain forms, some questions or parts of questions were not completed, as indicated here by captions or footnotes. Questions for which delegates indicated a positive response but could not provide further details (e.g., numbers) include a description of how that was recorded.

Question 1, The Companies: As expected from a European conference, European companies are the most strongly represented; they include all the small companies (<50 employees). Of those, four have only a primary location; two have other locations (both in the United States). Companies involved in early development and production are represented similarly, whereas fewer companies are involved in contract testing.

Question 2, Product Type and Number of Companies with Products of Each Type and in Each Stage of Development: This question asked for the number of products at each stage, if known. The majority of responses did not indicate numbers, so results are reported here as the number of companies with products at each stage.

Companies with therapeutic biotechnology products are the most highly represented. A large proportion of products are in late-phase development or already on the market. Vaccines are not specifically listed.

Question 3A, Importance Assigned to the Bioassay; Question 3B: Are adequate resources assigned to It? Management, regulatory, and QA personnel are all reported as generally placing at least medium to high importance on the bioassay, with QA the highest, followed by regulatory. This sequence may reflect the degree to which different departments are directly involved with the use of bioassays and derived data. The affiliation of each respondent to one of the departments, which was not recorded, could also be a factor in the perception of importance assigned by that department.

A small majority (58%) of respondents reported that resources for bioassays were inadequate. This did not seem to depend particularly on company size.

Question 4, Bioassays — What Types and When Are They Developed and Used? Except for the column reporting total numbers of established assays, figures indicate the number of

companies responding positively in each category. Seven companies report using various assay types but not the number of assays per type: These were entered as one assay per type, and the figures may therefore underrepresent the true number of different assays used. Four companies report a total of seven assay types as used at various stages, which are not described as established.

Few responses are given for preference in assay type (whether an assay type is preferred or used only in the absence of an alternative). Many companies do not indicate for one or more of their assays the use or stage of product development when the assay is developed or used. Seven companies report using late-stage readout, early-stage readout, or cell-binding assays but do not report using cell lines or primary cells. The number of companies using primary cells and/or cell lines and the number of primary cells and/or cell lines used thus is probably higher than shown. The assay described in row "J" would be expected to use primary cells or a cell line, but because it does not specify which, that has been left separate.

Cell-line–based assays and immunoassays are the most commonly used types and represent the greatest number of established assays. Out results suggest that most of the companies using a binding assay or immunoassay also use a functional bioassay. One diagnostic company reported the immunoassay as the only type it used. Two companies report using "cell lines" without specifying whether their assays are functional. All others using an immunoassay or binding assay reported also using functional assay types.

The stage of product development (preclinical to P3) at which bioassays are developed is reported as the number of companies developing each bioassay type, which does not necessarily reflect the total number of bioassays developed at each stage. The majority of reports show assay development at preclinical stage, but there is still some development at Phase 3.

Question 9: Importance attributed to various criteria considered in selecting a bioassay[1]

Criteria for Choice of Bioassay		Zero	Low	Medium	High	NA
Availability of	Cells	1	3	2	28	1
	Reagents	1	8	15	10	1
	Critical reagents		3	7	25	1
	Equipment		8	14	14	
	Similar assays used		6	10	15	3
	In-house technical advice		12	14	7	
	In-house statistical advice	4	14	9	4	1
	In-house regulatory advice	7	12	8	4	1
	External technical advice	2	16	10	2	1
	External statistical advice	6	18	4	3	
	External regulatory advice	7	12	6	4	3
Development	Resources available		5	11	17	1
	Time		2	16	16	1
	Cost		6	20	8	1
	Validation	1	4	16	10	1
Routine Running	Analysis time	1	6	14	15	
	Cost	2	9	14	9	
	Infrastructure	1	8	19	7	
	Labor	2	7	15	10	
Performance	Intra-assay variability	1	2	18	16	
	Intermediate precision	1	2	15	19	
	Robustness	1	2	16	19	
	Ruggedness	2	3	16	12	
	Accuracy	1	1	17	18	
	Specificity	1	2	15	20	
	Sensitivity	3	7	15	12	
	Trends	2	1	20	11	1
Closeness to in vivo MOA			2	10	10	3
Changing Bioassay System		2	4	9	4	4
Transfer of Bioassay	In-house		9	13	8	3
	To contract laboratories	2	7	12	6	4
	Demonstrating comparability		7	10	13	1
Regulators		1	4	13	9	5
Customers			6	7	6	7

[1] 16 complete responses, 14 with 3 or fewer missing, 7 with 4 or more missing, 3 blank of which 1 specifies NA because selection of the bioassay is not his or her own choice

Question 5, Is There a Consistent Strategy of Using Certain Bioassay Types at a Specific Product Development Phase? Responses indicate that about one-third of companies have a consistent strategy of using certain bioassay types at specific product development phases, whereas a similar proportion have a strategy with partial consistency. The question concerned only consistency of strategy (rather than decisions made case by case). It did not distinguish between strategies for maintaining the same bioassay type through product development and those for progressing to different assay types with stages of product development. The degree of consistency does not appear to depend particularly on company size.

Question 6, The Purpose for Which the Bioassay Is Used: Delegates were asked that only those using bioassays for GMP purposes complete this question.

Lot-release and stability testing are the most commonly reported uses of

Question 10: Issues causing concern in bioassays

Criteria for Choice of Bioassay		Zero	Low	Medium	High	NA
Availability of	Cells	2	6	3	17	
	Reagents	2	8	9	6	
	Critical reagents	1	2	6	17	
	Equipment	2	7	9	7	
	Similar assays used	4	7	6	5	3
	In-house technical advice	2	10	6	6	1
	In-house statistical advice	1	10	6	8	2
	In-house regulatory advice	4	9	4	6	3
	External technical advice	6	10	3	3	5
	External statistical advice	7	7	3	6	4
	External regulatory advice	7	7	2	4	5
Development	Resources available	1	3	10	12	1
	Time	1	2	9	12	1
	Cost	1	10	10	6	1
	Validation	1	5	10	9	
Routine running	Analysis time	3	5	10	7	
	Cost	3	7	6	7	
	Infrastructure	1	6	12	4	1
	Labor	1	4	12	7	
Performance	Intra-assay variability	1	3	6	13	
	Intermediate precision	1	1	10	12	
	Robustness	1	2	8	13	
	Ruggedness	1	3	12	8	
	Accuracy	1	4	9	10	
	Specificity	1	7	6	11	
	Sensitivity	2	7	8	7	
	Trends	1	3	9	8	1
Closeness to in vivo MOA		1	3	7	7	2
Changing bioassay system		2	2	6	6	2
Transfer of bioassay	In-house	2	7	5	8	2
	To contract laboratories		5	7	3	8
	Demonstrating comparability		4	7	10	3
Regulators			4	7	12	6
Customers		1	4	3	3	8
Other						

[1] 11 complete responses, 11 with 3 or fewer missing, 9 with 4 or more missing, 9 blank

bioassays. Immunogenicity and impurity testing are the least frequently reported uses. Less frequently reported uses may indicate either less testing for those purposes or the use of other methods.

Question 7, Number of Bioassay Types per Project: This question aimed to find whether multiple assay types were used for a given project at each stage of development. It also examined whether different assay types were used concurrently or whether companies switch from one type to another. For the first part, the answer requested the number and identification of assay types from Question 4. Of the 26 responses, many gave only a number or an assay type. Because it appeared that some replies referred to the total number of assays rather than assay types, no meaningful results can be reported.

Question 8, Would Delegates Like to Replace Functional Bioassays? Delegates were asked that only those using bioassays for GMP purposes complete this question.

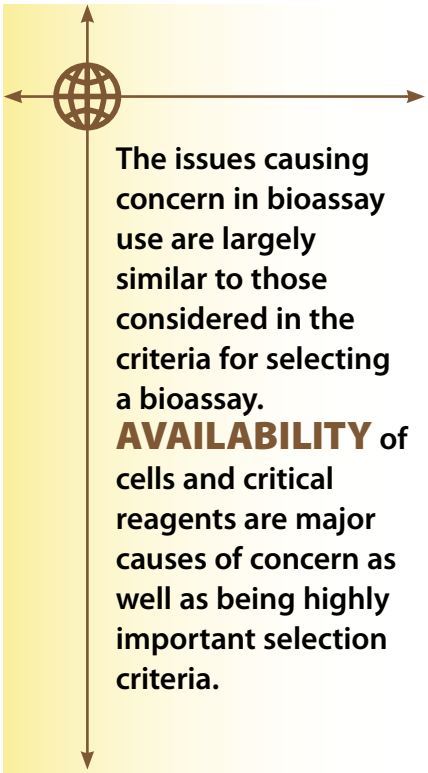
Over half of respondents (59%) would like to replace some or all of their existing functional assays with binding or physicochemical ones. More than three-quarters (81%) would prefer to replace functional assays for future products. Those specifying replacement assays demonstrated a preference for binding assays over physicochemical assays (72% for existing assays, 66% for future products). It must be noted that this question asks whether people *would prefer* to replace functional assays and not whether they actually intend to do so.

Question 9, Importance Attributed to Various Criteria Considered in Selecting a Bioassay: To permit distinction between zero impact, not applicable (NA), and incomplete answers, respondents were asked to select one of the five categories for each item rather than leaving any blank.

No large difference in the number of responses appeared between the beginning and end of question 9; any such difference could have influenced the apparent relative importance attributed to the various criteria. The mean number of responses per criterion for the first half (criteria 1–17) is 33.7 and for the second half (criteria 18–34) is 33.1. There are, however, noticeable criterion-specific differences in the number of responses. For example the assay-performance–related criteria (20–27) show a mean of 36.5 responses, range 33 to 38, whereas reported criteria concerning closeness to in vivo MOA (method of action) has 25 responses.

The most widely reported criteria considered of high importance in selecting a bioassay are the availability of cells and critical reagents. The various criteria for assay performance are of high or medium importance overall.

Availability of in-house or external statistical or technical advice is generally only of low or medium importance. Closeness to in-vivo mode



Question 11: Stability of the in-house bioassay reference standard[1]

A) Measures to Increase Stability	
None	3
Low temperature	30
Lyophilize	8
Inert gas	2
Other	1 (humidity)
B) Monitoring Stability	
None	1
Against external standard	18
Against similar preparation	15
Accelerated degradation-prediction	6
Against low temperature storage	10
Absolute measurement (IC50, kD)	8
Physicochemical test	15
Other	1 (trending)

[1] 31 responses to both A and B; 2 responses to A only; 4 responses to B only; 3 blank

Question 12: Consultation with regulators[1]

A	Stage		Helpful?
	Preclinical	14	10
	P1	5	3
	P2	4	1
	P3	4	3
	Ongoing	10	7
B	**Yes**	**No**	**NA**
Consistency between consultations	4	4	8
Consistency between regulators	1	6	10
Regulators' Questions			
Specificity	4	1	8
Validation	7		7
Stability indicating	4	1	7
Other (precision, robustness, sensitivity, cut-off)	2		

[1] 14 responses to a A and B; 4 to A only; 2 to B only; 3 whole question NA; 1 "don't know,"16 blank

of action is answered by relatively few, but classed as high or medium importance by those who do answer.

Question 10, Issues Causing Concern in Bioassays: As with question 9, respondents were asked to select one of five categories for each item (rather than leaving any blank) to permit distinction of zero impact, NA, and incomplete responses. Question 10 has slightly fewer responses per item (mean 23.2) to the second half of the question (items 18–34) than for the first half (mean 25.8), which may affect slightly the perceived relative importance of different criteria. There is, however, greater variation between individual questions (mean number of responses ranging from 18 to 28).

Issues causing concern in the use of bioassays are largely similar to those considered in the criteria for selecting a bioassay. Availability of cells and critical reagents are major causes of concern as well as being highly important selection criteria. The availability of advice — technical, statistical, and regulatory — features slightly more prominently in the high importance category for causes of concern than it does in the criteria for selecting a bioassay.

Question 11, Stability of In-House Bioassay Reference Standards: Low temperature is the most widely reported measure used to increase the stability of in-house reference standards. **(Note:** One laboratory used all listed methods for monitoring stability.) Lyophilization is used by 27% of respondents, but this does not necessarily indicate that 27% of standards are or can be lyophilized.

The laboratory reporting no monitoring of stability of reference standards used low temperature to increase stability. This respondent reported its business as production of therapeutic products and ancillary to therapeutic products and used a variety of assay types.

Question 12, Consultation with Regulators: The majority of consultations with regulators are reported as being helpful. There is only a small sample size here, but the responses suggest that there may be more consistency between consultations with a given regulator than between regulators.

Question 13, Statistical Support: In-house statisticians or biostatisticians are found mainly in larger companies, whereas small companies are the most likely to have no statistical support at all. An in-house biostatistician, statistician, or consultant biostatistician was reported in 15 responses, which reported that no statistician is involved in bioassay design or analysis. Commercial statistical packages are reported as being used in 41% of companies, and 16% report validation of packages.

Question 13: Statistical support (37 responses)

Statistical Support	
None	7
In-house statistician	15
In-house biostatistician	5
Consultant biostatistician	10
Statistician involved in assay design	5
Statistician involved in analysis	9
Use of commercial packages	15
In-house validation of packages	6

Company Size	No Statistical Support	In-house Statistician or Biostatistician
<50	4	—
50–249	1	3
≥250	2	13

Identifying Issues of Widespread Concern

Most of the data are reported in raw form so here readers can examine issues of particular interest to them. In tablulation of our data from the survey forms, some links are inevitably lost. Readers particularly interested in specific links or correlations are invited to contact us.

Caution must be exercised in interpreting or extrapolating these results. The survey was conducted on a population of limited size and not representative of the total global biopharmaceutical industry. There is geographical bias in the location of the organizations represented. Factors such as existing expertise and financial resources may influence the type and size of companies sending delegates to the conference. Individual delegates may not have knowledge of all relevant company activities.

Allowing for its limitations, this survey is valuable in providing unique data on the current use of bioassays in the biopharmaceutical industry. It identifies issues of widespread concern and indicates the degree to which users wish to change from functional bioassays to other systems. These data may assist those seeking solutions to common problems through collaborative ventures and support from government and industrial development initiatives.

More detailed information would have benefited many of our survey questions. However, that would have increased the survey length and made it too onerous to complete. A future approach might be to select one or two of the topics identified here for more detailed consideration.

Acknowledgments

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