Overview

The term “clinical proteomics” is much in vogue these days. Conferences are held to discuss its promise, headlines in trade publications announce the “imminent arrival” of the technology and a host of market research reports attempt to predict the future size of this nascent market.

But what exactly is “clinical proteomics?” BioInformatics, LLC set out to explore this issue knowing that while two-dimensional gel electrophoresis (2D PAGE) is a commonly used technique for protein analysis, newer and faster methods such as protein arrays would need to be used in a clinical environment. What survey research uncovered is that not only are relatively few researchers employing protein arrays in their disease studies, this select few are not yet able to clearly articulate their needs in terms of signal intensity, variation and detection. And while a majority of those currently using traditional 2D PAGE and mass spectroscopy plan to move to microarrays in the next 12 months, they are already aware of the challenges posed by inadequate reference databases and the difficulty of obtaining clinical samples.

Early markets are comprised of innovators and visionaries—often they are influential researchers with reputations for innovation and for pushing existing technologies to their limits. Their work tends to play an important role in shaping the product expectations of the “early adopters” who will follow them, but they themselves readily admit their requirements are not yet truly understood. Such is the case with the tools and techniques of clinical proteomics. For suppliers hoping to dominate this technology, which could profoundly change some segments of the clinical testing market, understanding the current experiences of visionaries and monitoring how their needs change over time is critical.

Unique Elements of This Report

- Based on the opinions of over 300 scientists whose protein-profiling studies focus on the diagnosis or prognosis of human disease
- Up-to-date data collected between July 29 and August 19, 2003
- Over 50 full-color analytical tables and charts
- Approximately 10 cross-tabulations
- More than 50 comments from scientists exploring the clinical proteomics frontier that detail the barriers they feel must be overcome
- Delivered quickly as an Excel file allowing you to import the data into your presentations to senior management, staff, partners and investors

Clinical Proteomics: A First-Glance Market Report confirms that the effectiveness of clinical proteomics will hinge on two technological components: rapid, multiplex protein detection assays and data analysis systems to assimilate vast amounts of protein expression data from healthy and diseased individuals into clinically relevant data sets. Based on a detailed survey of proteomic researchers, this First-Glance Market Report identifies the research objectives, methodologies, assay parameters and technological limitations that are currently associated with disease-based protein-profiling studies. In addition, these visionaries provide their thoughts on the disease applications, assay formats and technical standards for future protein-profiling assays that will be used for disease prognosis or diagnosis in a clinical setting. For suppliers of products for protein-profiling research, as well as for companies that need to stay abreast of the latest developments in clinical testing, this report provides a wealth of information about the present state and future directions of clinical proteomics.

What is a First-Glance Market Report?

When a market is in an embryonic state, traditional techniques for understanding its size, growth and the needs of customers are found wanting. There is simply too little consensus in terms of definitions, standards, product mix and expectations to measure and use in planning business strategies. Yet understanding the current experiences of today’s visionaries and monitoring how their needs change over time is key for suppliers hoping to dominate a new, revolutionary technology.

BioInformatics’ First-Glance Market Reports are designed to meet the needs of strategic planners for time-sensitive, exploratory research. Unlike our premier market reports that provide statistically significant data and extensive analysis of measurable markets, First-Glance Market Reports deliver data right from an early market’s visionaries to their counterparts in industry—quickly and at an affordable cost. First-Glance Market Reports are also based on questionnaires specifically designed to support repeat surveys so that user needs and changing expectations of performance can be measured over time.
Scientific Advances Prompting This Study

Most currently used disease assays focus on a limited repertoire of biomarkers such as PSA for prostate cancer or cardiac troponin for myocardial infarction. While there has been significant progress in the development of rapid immunological assays for individual antigens, many diseases and disorders cannot be diagnosed accurately with these types of tests due to a lack of reliable protein biomarkers.

The recent completion of the human genome sequence and the emergence of sophisticated new technologies for identifying DNA polymorphisms offer the hope that genetic testing will greatly expand the range of effective diagnostic assays. However, diseases such as cancer can arise from a variety of mutations, many of which will fall beyond the scope of common genetic tests.

Gel electrophoresis is still the most common assay used to confirm protein-profiling data.

Rather than focusing on genetic alterations that may lead to disease, many researchers believe that identifying changes in protein expression patterns, commonly referred to as protein-profiling, is the most accurate way to identify diseases in their early stages and to determine the most effective courses of treatment.

Clinical proteomics refers to the application of protein-profiling techniques for disease prognosis, diagnosis or treatment monitoring. In contrast to standard protein-based clinical assays, which typically measure single analytes, protein-profiling technologies simultaneously analyze hundreds or even thousands of proteins from a given sample. Protein-profiling potentially can achieve much higher levels of prognostic or diagnostic accuracy compared to analyses of individual proteins, which often correlate imperfectly with the presence of a disease or cannot be detected until the disease has reached an advanced stage.

Two-dimensional gel electrophoresis (2D PAGE) and mass spectrometry (MS) remain the primary tools for protein-profiling. 2D PAGE separates proteins based on their charges and molecular weights. While this technique can identify hundreds of proteins in a single assay, it has a number of limitations such as large sample input requirements, lack of reproducibility, intensive labor requirements and difficulty in protein identification. Some of these challenges are being addressed by recently developed technologies, such as sophisticated gel scanners, image analysis software and robotic devices. Also, “zoom” gels with narrow pH ranges for isoelectric focusing are becoming increasingly popular because they offer higher resolution and sensitivity compared to standard 2D PAGE.

41% of those scientists performing protein-profiling do so with the objective of identifying protein expression patterns associated with diseases.

Another relatively new technique, difference gel electrophoresis (DIGE), has greatly extended the utility of 2D PAGE for comparing protein expression patterns between two samples. Prior to electrophoresis, proteins in each sample are labeled with different fluorescent dyes, frequently Cy3 and Cy5. By co-resolving the two labeled protein populations within the same gel, researchers can identify individual proteins that are expressed at different levels in the two populations.

To identify proteins in 2D PAGE gels, researchers often use MS. After electrophoresis, individual spots are excised, proteolytically digested and analyzed either by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) or by electrospray ionization-based MS. Protein identities are determined from the charge-to-mass ratios of ionized peptides.

Objectives of This Study

- Identify the commonly used methods and applications for protein-profiling
- Define the experimental parameters and levels of usage that are associated with the use of protein arrays/chips for protein-profiling
- Detail the problems/limitations associated with the use of protein arrays/chips
- Ascertaining the most likely disease applications and assay formats for future clinical protein-profiling assays
- Describe the performance standards that should be considered when developing protein-profiling assays for the clinical lab
Methodology

310 scientists completed a 39-question survey conducted by BioInformatics, LLC (Arlington, Virginia, USA) between July 29 and August 19, 2003.

The electronic questionnaire was fielded to registered members of The Science Advisory Board. BioInformatics sponsors The Science Advisory Board, an online community of more than 16,000 scientists, physicians and healthcare professionals from around the world. The Science Advisory Board is divided into two panels (Research and Clinical) and “convenes” regularly via the World Wide Web (www.scienceboard.net) to voice their opinions on a wide variety of issues relating to biomedical research and clinical technologies. These experts—representing all aspects of the life sciences and medicine—have agreed to make themselves available to participate in our online research activities. The Science Advisory Board members who participated in this study were drawn from both panels and supplemented by additional qualified life scientists.

Three skip patterns were set up in the body of the questionnaire to direct the respondents to the questions that best reflect their experience in using 1) protein-profiling studies as they relate to the diagnosis or prognosis of human disease (Question 6), 2) protein arrays (Question 7) and 3) the method of detection employed when analyzing protein arrays (Question 20).

Aside from 2D PAGE, other separation techniques, such as liquid chromatography (LC) or capillary electrophoresis (CE) are also used in conjunction with MS for protein-profiling. To resolve complex protein samples such as whole cell or tissue extracts, multiple separation methods may be combined. For example, the technique known as multidimensional protein identification technology or “MudPit” uses strong cation exchange followed by reverse phase chromatography prior to MS.

Another method for identifying differentially expressed proteins in mixed samples, similar to DIGE described above, is isotope-coded affinity tagging (ICAT). With this technique, cysteine residues in two protein samples are labeled with different carbon isotopes. Following LC, differentially expressed proteins are identified by using MS to quantify the relative ratios of peptides labeled with the heavy and light isotopes. Although MS is widely used for protein-profiling, like 2D PAGE, it suffers from a number of drawbacks such as the need for extensive sample preparation, cost and difficulties with data analysis.

Protein Chips and Arrays

Confronted with the limitations of conventional protein-profiling methods, scientists have looked to recent advances in the field of genomics for technologies that can be adapted to improve the speed and sensitivity of proteomic analyses. In particular, the rapidly growing popularity of DNA microarrays has led to the development of microarrays and chips for protein research.

Rather than using DNA probes, protein arrays/chips employ matrices of peptides, antibodies, aptamers or chromatographic surfaces that can selectively capture proteins from complex mixtures. In some cases, the same instruments that are used to manufacture and analyze DNA microarrays can also used for protein array studies. However, proteins differ considerably from nucleic acids, and there are added challenges to working with protein arrays/chips compared to DNA microarrays.

One of the biggest difficulties with protein arrays relates to the attachment of protein probes to solid surfaces. Unlike DNA, which is essentially a one-dimensional molecule, proteins must retain their three-dimensional structures to interact with other proteins. Thus, researchers have struggled to develop surface

Demographics

Market Segment

<table>
<thead>
<tr>
<th>Segment</th>
<th>Respondents</th>
<th>%</th>
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<tbody>
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<td>Academic</td>
<td>127</td>
<td>41%</td>
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<tr>
<td>Pharmaceutical/Biotechnology</td>
<td>71</td>
<td>23%</td>
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<tr>
<td>Hospital or University Medical Center</td>
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<td>18%</td>
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<tr>
<td>Government</td>
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<td>5%</td>
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<tr>
<td>Private Research</td>
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<td>5%</td>
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<tr>
<td>Contract Research</td>
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<td>4%</td>
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<tr>
<td>Medical Device/Diagnostics</td>
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<tr>
<td>Commercial Testing Lab</td>
<td>3</td>
<td>1%</td>
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<tr>
<td>Healthcare Network/Facility</td>
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<td>1%</td>
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<tr>
<td>Group/Private Practice</td>
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<td>&lt;1%</td>
</tr>
<tr>
<td>Managed Care</td>
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<td>&lt;1%</td>
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Job Position

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<th>Position</th>
<th>Respondents</th>
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</thead>
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<tr>
<td>Principal Investigator</td>
<td>73</td>
<td>24%</td>
</tr>
<tr>
<td>Staff Scientist</td>
<td>62</td>
<td>20%</td>
</tr>
<tr>
<td>Lab Director/Supervisor/Coordinator</td>
<td>51</td>
<td>16%</td>
</tr>
<tr>
<td>Post Doctorial Fellow</td>
<td>38</td>
<td>12%</td>
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<tr>
<td>Graduate Student/Research Assistant</td>
<td>33</td>
<td>11%</td>
</tr>
<tr>
<td>Professor/Teacher</td>
<td>24</td>
<td>8%</td>
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<tr>
<td>Department Head</td>
<td>9</td>
<td>3%</td>
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<tr>
<td>Physician</td>
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<td>2%</td>
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<tr>
<td>Laboratory Technician</td>
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<td>1%</td>
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<tr>
<td>Other</td>
<td>4</td>
<td>1%</td>
</tr>
<tr>
<td>Quality Assurance/Quality Control</td>
<td>3</td>
<td>1%</td>
</tr>
<tr>
<td>Administrator</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>IS Manager/Specialist</td>
<td>1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Medical Technologist</td>
<td>1</td>
<td>&lt;1%</td>
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Geographic Region

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<td>Europe</td>
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<td>23%</td>
</tr>
<tr>
<td>Asia</td>
<td>19</td>
<td>6%</td>
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<tr>
<td>Australasia/Pacific</td>
<td>9</td>
<td>3%</td>
</tr>
<tr>
<td>Central/South America</td>
<td>6</td>
<td>2%</td>
</tr>
<tr>
<td>Africa</td>
<td>2</td>
<td>1%</td>
</tr>
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</table>
Increased assay sensitivity is the top technological improvements scientists say needs improvement before protein-profiling assays can be used for disease diagnosis/prognosis in a clinical setting.

Aside from planar arrays, other formats for protein arrays/chips include liquid bead arrays and microfluidic lab-on-a-chip assays. Also, there are several different methods for detecting analyte proteins that are captured on protein arrays/chips. Fluorescence scanners are the most common detection devices, but other technologies such as MALDI-TOF MS and surface plasmon resonance are frequently used.

Protein-profiling in the Clinic

Although protein-profiling is currently used for a variety of research applications, the technology has not yet been adopted as a routine method for clinical testing. This is due to several factors, including the aforementioned limitations with 2D PAGE, MS, and protein array/chip assays. In addition, it is difficult at present to base clinical decisions on protein-profiling data due to the limited availability of information on protein expression patterns in healthy and diseased subjects.

Nevertheless, results from several clinical research groups suggest that protein-profiling technologies may offer significant improvements over current assays for disease prognosis and diagnosis. For example, the Clinical Proteomics Initiative, a joint venture between the National Cancer Institute and the Food and Drug Administration, recently used protein chips coupled with mass spectrometry to compare blood serum protein-profiles between 50 women with known ovarian cancer and 50 women without disease. After using an artificial intelligence algorithm to process and store the resulting data, the scientists were then able to identify correctly ovarian cancer in 50 out of 50 women who had the disease and to identify 63 out of 65 unaffected women as being non-malignant using blinded samples. Most importantly, the technology was shown to be effective for identifying patients with early stage cancers, which are most treatable (http://ccr.nci.nih.gov/tech_initiatives/clinical_proteomics.asp).

Further advances in clinical proteomics will require improvements not only in laboratory technologies but also in the databases and algorithms that store and interpret protein-profiling information. Researchers need greater access to clinical specimens, and standards must be adopted to ensure that laboratories can easily exchange and compare data. As these improvements are implemented, the potential uses for clinical proteomics will likely extend beyond cancer diagnostics to include applications such as antibiotic and drug sensitivity profiling, monitoring stress and cell death pathways for toxicology purposes, and measuring protein expression patterns that are characteristic of cardiovascular disease, metabolic disorders and a variety of other maladies.

Of the scientists who have adopted protein array technology for their protein-profiling assays, 49% obtain their arrays from a commercial supplier, with Ciphergen and BD Clontech cited as the leading vendors.
Question 1
Respondents who perform protein-profiling assays proceeded to take the survey. (N=428)

Question 6
Respondents whose protein-profiling studies focus on the study of human disease continued the survey. (N=310)

Question 7
Respondents who use protein-arrays/chips for protein-profiling studies proceeded to Question 8. (N=78)

Question 7
Respondents who do not use protein-arrays/chips for protein-profiling studies proceeded to Question 29. (N=232)

Questions 8 through 28
Only those respondents who apply protein-profiling techniques to the study of human disease AND use protein arrays answered these questions. (N=78)

Question 29 through 36
All respondents who apply protein-profiling assays to the study of human disease answered these questions. (N=310)
Questionnaire

For your reference and convenience, a copy of the survey used to conduct this study can be found on Pages 6–13.

For the purposes of this survey, the term 'protein-profiling' refers to techniques or methods that can simultaneously detect many different proteins or identify broad patterns of protein expression in a biological sample using a single assay.

1. Do you perform protein-profiling assays? (choose only one)
   - Yes, I began... 0 to 6 months ago
   - No, but I plan to within the next... 7 to 12 months ago
   - No, and I don’t plan to PRIMARILY because of...
     - Cost
     - Lack of expertise
     - Lack of instrumentation
     - Not developed for research needs
     - Not developed for clinical needs
     - Unnecessary for current objectives
     - Other (please specify) ___________

   Respondents who do not use protein-profiling assays exited the survey.

2. What is your PRIMARY objective for protein-profiling? (choose only one)
   - Discovering biomarkers for standard disease assays
   - Discovering biomarkers for standard toxicology assays
   - Drug target discovery
   - Drug target validation
   - Evaluation/monitoring of therapeutic treatments
   - Identifying protein expression patterns associated with diseases
   - Identifying protein expression patterns associated with toxicants
   - Other (please specify) ___________

3. Where do you perform the MAJORITY of your protein-profiling assays? (choose only one)
   - Basic research laboratory
   - Clinical research laboratory
   - Clinical testing laboratory
   - Industrial research laboratory
   - Other (please specify) ___________

4. Which type(s) of samples do you use for protein-profiling? (check all that apply)
   - Amniotic fluid
   - Cerebrospinal fluid
   - Gastric fluid
   - Saliva
   - Serum/plasma
   - Solid human tissue
   - Sputum
   - Stool
   - Synovial fluid
   - Urine
   - Whole blood
   - Other (please specify) ___________

5. Which type(s) of assays do you use to confirm protein-profiling data? (check all that apply)
   - DNA polymorphism analysis
   - ELISA/EIA
   - Enzyme activity assay
   - Gel electrophoresis
   - Gene expression profiling using DNA microarrays
   - Gene expression profiling using non-array-based methods
   - Histology with tissue microarrays
   - Immunoblotting
   - Karyotype analysis/FISH
   - Lateral flow immunochromatography
   - Solid phase immunoassay (e.g., "dipstick")
   - Other (please specify) ___________
6. Are your protein-profiling studies focused on the diagnosis or prognosis of human diseases or conditions? (choose only one)

☐ Yes, I am developing or testing prognostic/diagnostic assays PRIMARILY for...
  ☐ Cancer (please specify type) ____________
  ☐ Age-related illness
  ☐ Alcohol/drug addiction
  ☐ Allergy
  ☐ Autoimmune disease
  ☐ Cardiovascular disease
  ☐ Developmental defects
  ☐ Infectious disease
  ☐ Metabolic/hormonal disorder
  ☐ Muscular/skeletal disease
  ☐ Neurological disorder
  ☐ Other disease (please specify) ____________

☐ Yes, I am developing or testing protein-profiling assays that can be used for multiple diseases

☐ No, I am not developing or testing prognostic or diagnostic protein-profiling assays for human diseases

**Respondents who do not develop or test prognostic/diagnostic assays exited the survey after Question 7.**

7. Which method do you PRIMARILY use for protein-profiling? (choose only one)

☐ Mass-spectrometry without protein arrays/chips

Protein arrays/chips
  ☐ Chromatographic matrix chip (e.g., Ciphergen)
  ☐ Liquid bead array (e.g., Luminex)
  ☐ Microfluidic lab-on-a-chip
  ☐ Printed/spotted array
  ☐ Two-dimensional polyacrylamide gel electrophoresis
  ☐ Other (please specify) ____________

**Respondents who do not use protein arrays/chips for protein-profiling proceeded to Question 29.**

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**Protein Arrays/Chips**

8. On average, how many arrays/chips do you use per month for protein-profiling assays? (please specify)

__________________

9. How will the number of arrays/chips that you use for protein-profiling change over the next twelve months? (choose only one)

☐ Increase by
  ☐ Less than 20%
  ☐ 20% to 39%
  ☐ 40% to 59%
  ☐ 60% to 79%
  ☐ 80% to 100%
  ☐ More than 100%

☐ Decrease by
  ☐ Less than 20%
  ☐ 20% to 39%
  ☐ 40% to 59%
  ☐ 60% to 79%
  ☐ 80% to 100%
  ☐ More than 100%

☐ Stay the same

☐ Not sure
10. What is the PRIMARY source of the arrays/chips that you use for protein-profiling? (choose only one)
☐ Commercial supplier (choose a supplier)
  - BD Clontech
  - Hypromatrix
  - Biacore
  - Molecular Staging
  - Biocat
  - Panomics
  - Biochain
  - RayBiotech
  - Ciphergen
  - Zymyx
  - Biocat
  - Molecular Staging
  - Biochain
  - RayBiotech
  - Ciphergen
  - Zymyx
  - HTS Biosystems
  - Create in my own lab
  - Custom made, non-commercial source (e.g., core facility, collaborator)
  - Other (please specify) ____________

11. What is the PRIMARY capture agent on the arrays/chips that you use for protein-profiling? (choose only one)
Biomolecule capture agent
☐ Antibody or antibody fragment
☐ Aptamer
☐ Carbohydrate
☐ Lipid
☐ Peptide
☐ Protein (non antibody)
☐ Chromatographic surface
☐ Small molecule capture agent
☐ Other (please specify) ____________

12. On average, how many unique capture agents or chromatographic surfaces are on a single protein array/chip that you use for protein-profiling? (choose only one)
☐ Not applicable
☐ Less than 10
☐ 10 to 49
☐ 50 to 99
☐ 100 to 249
☐ 250 to 500
☐ More than 500

13. Do you typically process biological samples prior to analysis with protein arrays/chips? (choose only one)
Yes, I use... (check all that apply)
☐ Affinity chromatography
☐ Cell lysis/homogenization
☐ Fluorescent labeling
☐ Gradient centrifugation
☐ Ion exchange chromatography
☐ Isotopic labeling
☐ Non-gradient centrifugation
☐ Proteolytic cleavage
☐ Size-exclusion chromatography
☐ Other (please specify) ____________
☐ No, I apply unprocessed biological samples directly to protein arrays/chips

14. On average, what volume of sample do you use for a single protein-profiling assay with arrays/chips? (choose only one)
☐ Less than 1 µl
☐ 1 to 19 µl
☐ 20 to 39 µl
☐ 40 to 59 µl
☐ 60 to 79 µl
☐ 80 to 100 µl
☐ More than 100 µl

To obtain the survey results, see page 14.
15. With the arrays/chips that you use for protein-profiling, what is the average limit of detection for proteins in biological samples? (choose only one)

- Not sure
- Less than 1 pg/ml
- 100 pg/ml
- 500 pg/ml
- 1 ng/ml
- 100 ng/ml
- 500 ng/ml
- 1 µg/ml
- More than 1 µg/ml

16. What is the linear dynamic range (in logarithmic units) of the PRIMARY array/chip-based assay that you use for protein-profiling? (choose only one)

- Not sure
- Less than 2
- 2 to 4
- 5 to 7
- 8 to 10
- More than 10

17. Based on comparisons between samples from diseased and healthy individuals tested in your laboratory, what is the average SENSITIVITY (i.e., percentage of diseased samples that test positive) of the array/chip-based assay that you most commonly use? (choose only one)

- Don't know
- Less than 50%
- 60 to 69%
- 70 to 79%
- 80 to 89%
- 90 to 94%
- 95 to 99%
- More than 99%

18. Based on comparisons between samples from diseased and healthy individuals tested in your laboratory, what is the average SPECIFICITY (i.e., percentage of non-diseased samples that test negative) of the array/chip-based assay that you most commonly use? (choose only one)

- Don't know
- Less than 50%
- 60 to 69%
- 70 to 79%
- 80 to 89%
- 90 to 94%
- 95 to 99%
- More than 99%

19. Overall, how satisfied are you with the performance characteristics of the protein arrays/chips that you use for disease diagnosis/prognosis? (choose only one)

<table>
<thead>
<tr>
<th>Very Satisfied</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4</th>
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<th>Not Satisfied</th>
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</tbody>
</table>
20. What is your PRIMARY method for detecting/analyzing proteins that you capture on arrays/chips? (choose only one)
- Fluorescence
- Luminescence
- Mass spectrometry
- Phosphorimaging
- Scintillation
- Surface plasmon resonance
- Other (please identify) ____________

Respondents who use mass spectrometry for detecting/analyzing proteins proceeded to Questions 25 to 28.

21. For the arrays/chips that you use for protein-profiling, what is the average variation in signal intensity between replicate spots on the SAME array/chip? (choose only one)
- Not applicable
- Less than 10%
- 10 to 19%
- 20 to 39%
- 40 to 59%
- 60 to 79%
- 80 to 100%
- More than 100%

22. What is the average variation in signal intensity between replicate spots on DIFFERENT arrays/chips that you use for protein-profiling? (choose only one)
- Not applicable
- Less than 10%
- 10 to 19%
- 20 to 39%
- 40 to 59%
- 60 to 79%
- 80 to 100%
- More than 100%

23. How do the limits of detection vary between different capture agents on the SAME array/chip? (choose only one)
- Not sure
- Less than 10X
- 10 to 99X
- 100 to 1,000X
- More than 1,000X

24. What are the top THREE problems/limitations that you have with the protein arrays/chips that you use for protein-profiling? (check only THREE)
- Accuracy of measurements
- Detecting high molecular weight proteins
- Detecting low concentrations of proteins
- Loss of capture agent activity during storage
- Obtaining capture agents for array/chip production
- Obtaining premade arrays/chips with desired capture agents/chromatographic surfaces
- Variable affinities among different capture agents
- Variable affinities between replicate capture agents on DIFFERENT arrays/chips
- Variable affinities between replicate capture agents on the SAME array/chip
- Other (please specify) ____________
### Mass Spectrometry Detection

25. How many different mass/charge ratios (m/z) do you measure in a single sample when performing protein-profiling with protein chips? (choose only one)

- [ ] Less than 1,000
- [ ] 1,000 to 9,999
- [ ] 10,000 to 49,999
- [ ] 50,000 to 100,000
- [ ] More than 100,000

26. What is the upper limit of mass resolution in the mass spectrometry instrumentation that you use for protein-profiling with protein chips? (choose only one)

- [ ] Less than 500 Da
- [ ] 500 to 999 Da
- [ ] 1,000 to 10,000 Da
- [ ] 10,000 to 50,000 Da
- [ ] 50,000 to 100,000 Da
- [ ] More than 100,000 Da

27. What is the accuracy of the mass spectrometry instrumentation that you currently use for protein-profiling with protein chips? (choose only one)

- [ ] Less than 1 ppm
- [ ] 1 to 9 ppm
- [ ] 10 to 49 ppm
- [ ] 50 to 100 ppm
- [ ] More than 100 ppm

28. What are the top THREE problems/limitations that you have when using mass spectrometry for profiling proteins captured on arrays/chips? (check only THREE)

- [ ] Accuracy of measurements
- [ ] Detecting high molecular weight proteins
- [ ] Detecting low concentrations of proteins
- [ ] Discriminating between peaks in mass spectrometry readouts
- [ ] Loss of capture agent activity during storage
- [ ] Obtaining capture agents for array/chip production
- [ ] Obtaining premade arrays/chips with desired capture agents/chromatographic surfaces
- [ ] Variable affinities among different capture agents
- [ ] Variable affinities between replicate capture agents on DIFFERENT arrays/chips
- [ ] Variable affinities between replicate capture agents on the SAME array/chip
- [ ] Other (please specify) ____________

- ▶ All of the respondents proceed to answer the rest of the survey (N=310).

29. What problems/limitations do you have with the protein-profiling assays that you specifically use for disease testing? (check all that apply)

- [ ] Difficulty obtaining clinical samples
- [ ] Lack of adequate reference databases for disease biomarker evaluation
- [ ] Limited linear dynamic range
- [ ] Sample preprocessing
- [ ] Sample quality
- [ ] Sample volume requirements
- [ ] Sensitivity for disease diagnosis/prognosis
- [ ] Signal-to-noise
- [ ] Specificity for disease diagnosis/prognosis
- [ ] Other (please specify) ____________
Future Clinical Applications

30. In your opinion, what disease or condition is the BEST candidate for a diagnostic or prognostic assay based on protein-profiling? (choose only one)
   - Cancer (please specify type) ____________
   - Age-related illness
   - Alcohol/drug addiction
   - Allergy
   - Autoimmune disease
   - Cardiovascular disease
   - Developmental defects
   - Infectious disease
   - Metabolic/hormonal disorder
   - Muscular/skeletal disease
   - Neurological disorder
   - Other disease (please specify) ____________

31. In your opinion, what is an acceptable level of SENSITIVITY (i.e., percentage of diseased samples that test positive) for a protein-profiling assay that will be used for prognosis/diagnosis of the disease/condition that you specified above? (choose only one)
   - Less than 50%
   - 60 to 69%
   - 70 to 79%
   - 80 to 89%
   - 90 to 94%
   - 95 to 99%
   - More than 99%

32. In your opinion, what is an acceptable level of SPECIFICITY (i.e., percentage of non-diseased samples that test negative) for a protein-profiling assay that will be used for prognosis/diagnosis of the disease/condition that you specified above? (choose only one)
   - Less than 50%
   - 60 to 69%
   - 70 to 79%
   - 80 to 89%
   - 90 to 94%
   - 95 to 99%
   - More than 99%

33. In your opinion, what type of protein-profiling system will be most applicable for diagnosis/prognosis of the disease/condition that you specified above? (choose only one)
   - Capillary electrophoresis/mass spectrometer
   - Liquid bead array/flow cytometer
   - Liquid chromatography/mass spectrometer
   - Microfluidic lab-on-a-chip system
   - Planar protein array/microarray scanner
   - Planar protein array/surface plasmon resonance detector
   - Protein chip/mass spectrometer
   - Two-dimensional gel electrophoresis/gel scanner
   - Two-dimensional gel electrophoresis/mass spectrometer
   - Other (please specify) ____________
34. What should be the sample volume requirement for the protein-profiling system that you specified above? (choose only one)
   - Less than 1 µl
   - 1 to 19 µl
   - 20 to 39 µl
   - 40 to 59 µl
   - 60 to 79 µl
   - 80 to 100 µl
   - More than 100 µl

35. What should be the throughput level (samples per day) for the protein-profiling system that you specified above? (choose only one)
   - Less than 100
   - 100 to 500
   - 500 to 1,000
   - More than 1,000

36. In your opinion, which THREE technological improvements are MOST needed before protein-profiling assays can be used for disease diagnosis/prognosis in a clinical setting? (check only THREE)
   - Greater diversity of capture agents for protein arrays
   - Higher affinity capture agents for protein arrays
   - Improved data analysis software
   - Improved reference databases
   - Increased assay sensitivity
   - Increased assay specificity
   - Increased batch-to-batch consistency of capture agents for protein arrays
   - Increased throughput capabilities
   - More efficient methods for producing capture agents for protein arrays
   - Reduced sample preparation requirements
   - Reduced sample volume requirements
   - Other (please specify) ____________

37. In your opinion, which THREE non-technical factors will MOST contribute to the adoption of protein-profiling assays for disease diagnosis/prognosis in a clinical setting? (check only THREE)
   - Availability of training for laboratorians
   - Cost of consumables
   - Cost of instrumentation
   - Insurance company acceptance
   - Multiple disease applications
   - Physician acceptance
   - Point-of-care applications
   - Recommendations by leading clinical researchers
   - Regulatory approval
   - Time-to-results
   - Other (please specify) ____________

38. In your opinion, how long will it be before protein-profiling assays are routinely used for disease diagnosis/prognosis in a clinical setting? (choose only one)
   - Less than 2 years
   - 2 to 4 years
   - 4 to 6 years
   - 6 to 8 years
   - 8 to 10 years
   - More than 10 years
   - Never
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