

# Proteomics and Vaccine Potency Testing

Louisa B. Tabatabai, PhD  
Respiratory Diseases of Livestock  
Research Unit  
National Animal Disease Center, ARS, USDA,  
Ames, Iowa

- What is proteomics
- Methodologies of proteomics
- Standardization in proteomics
- Pros and cons for using proteomics technology in vaccine testing in terms of advantages and disadvantages
- Some applications

# What is Proteomics

- The technology used to display the cell's proteome or protein components
- Provides a snapshot of the status of that cell or cells at the time of harvest or processing
- The principle methods used to separate the individual proteins are based on several intrinsic properties of the proteins

# Methods for display of proteins

- Basic methods used for the separation and display of proteins are
  - 2-dimensional electrophoresis
  - 2-dimensional high performance liquid chromatography (HPLC)
    - Variations are
      - MUDPIT (multidimensional protein identification technologies) separations Nature Biotechnol. 19,242-247 (2001)
      - ICAT (isotope coded affinity tag) separations J.Mass Spectr.37,1-14 (2002)

# Protein display

- Protein display in 2-D electrophoresis is based on two intrinsic protein properties
  - Isoelectric pH (pI) and molecular mass
- Proteins are displayed as a function of
  - pI in the X-dimension and
  - Mass in the Y-dimension
  - Density in the z-dimension

# Protein solubilization

- Proteins may be solubilized using various methods
  - Non ionic detergents, urea, thiourea, reducing agents Molloy Anal. Biochem. 280,1-10 (2000)
  - Ampholytes, to prevent protein from precipitating during isoelectric focussing
  - Treatment of bacterial membranes for complete protein extraction Molloy et al., Electrophoresis 22,1686-1696 (2001)

# First Dimension

- The first dimension is based on isoelectric focusing
  - Proteins that differ in pI as little as 0.01 pH unit or less are separated
  - Differences in pI are due to post-translational modification including
    - Glycosylation, phosphorylation, sulfation, adenylation, methylation, acetylation
  - “Isoforms” of proteins form horizontal “trains” of molecular species

# Second dimension

- Proteins are denatured *in situ* on the strip
- Strip is slid in place in ready-made gel cassette, molecular weight standards are applied, strip is sealed in place
- Gel is subjected to standard electrophoresis conditions

# Visualization

- Protein spots are visualized by staining procedures
  - Coomassie Brilliant Blue
    - Sensitivity approximately 0.1 to 0.5  $\mu\text{g}$  protein per spot and depends on the content of lysine and arginine
  - Sypro Ruby®
    - Sensitivity is from 2 to 10 ng per spot and depends on the content of lysine, arginine and histidine
    - Is a fluorescent ruthenium-chelate
    - Excitation: 280 nm, 450 nm; Emission at 610 nm
    - Measured with imaging systems equipped with lasers that emit at 450 nm, 473 nm, 488 nm or 532 nm

# Documentation

- Coomassie Blue stained gels
  - Flatbed scanner, saved as digitized image
- SYPRO Ruby®-stained gels
  - Laser scanner, saved as digitized image

# Analysis

- For both Coomassie Blue-stained gels and SYPRO Ruby®-stained gels
  - Software analysis packages available
  - Cost from \$12,500 to \$90,000
  - Assign molecular weight, relative density, can provide comparative densities of standards to test gels with respect to any spot
  - Gels can be archived for future reference

# Mass spectrometry

- If desired, the identity of individual spots can be determined
- The individual protein spots are sampled, subsequently digested in an automated digester with an enzyme (e.g. trypsin or chymotrypsin) to reduce its complexity to peptides
  - In some technologies the entire proteome is digested, followed by 2-dimensional HPLC to separate the peptides
- Resultant peptides are identified by mass spectrometry
  - According to mass (peptide mass finger printing)
  - Direct sequencing (collision-induced fragmentation)
- Database searching is available at the public database maintained at <http://prospector.ucsf.edu>
  - Programs such as MS-FIT and MASCOT
- Proprietary databases

# Standardization

- Factors that need to be considered include
  - Number of bacteria or viral particles for analysis
    - Bacterial culture can be adjusted to a predetermined density, lysed and subfractionated if required
  - The total protein amount to be used for analysis is determined by the size of the isoelectric focusing strip and the size of the gel Amersham Biosciences, #80-6429-60, Edition AC:

Strip	Gel	Protein
7 cm	10 cm	50-100 $\mu\text{g}$
11 cm	14 cm	100-250 $\mu\text{g}$
18 cm	24 cm	500-1000 $\mu\text{g}$

- To obtain improved reproducibility, both the isoelectric focusing strips and the gels are available commercially
  - Immobilized pH gradients are available in broad range and narrow ranges in the form of dehydrated plastic-backed polyacrylamide strips Görg et al., Electrophoresis 21, 1037-1053 (2000)
- SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel)
  - Standard, commercially-available gradient gels or homogeneous gels for the 10-cm and 14-cm sizes
  - Gradient gels provide improved resolution and reproducibility

# Advantages for using proteomics

- A 2-D gel provides a snapshot of the expressed proteins and can detect
  - Genetically modified bacterium or virus
  - Changes to environmental pressures such as repeated passage, altered medium composition
  - Differences between strains such as virulent and avirulent strains
  - Changes in antigenicity by using Western blots
- A reference library of 2-D gels can be established
- An extensive library of 2-D gels is available at <http://www.expasy.ch/ch2d> with links to many sites including human pathogens

# Disadvantages for using proteomics

- 2-D gel display of proteins depends on a number of factors
  - Dynamic range of proteins
    - low abundance proteins may not be observed, but can be remedied by
      - Subfractionation
      - Narrow range pH strips
  - Solubility of membrane proteins
    - Improved methods for solubilizing membrane proteins are available Molloy Electrophoresis 22,1686-1696 (2001)
  - Labor intensive

# Time commitment

- Grow and lyse culture
- Protein assay
- Sample preparation for isoelectric focusing, 10 min/sample
  - Accommodates 10 samples
- Overnight run, ~20 hr
- Second dimension, 1.5 hr/2 gels
- Stain, destain, 3-24 hr
- Documentation, 10 min/gel
- Analysis of gel, 2-4 hr

# Instrumentation

- Considerations
  - For the gel-based system the start-up cost is relatively low, but depends on
    - Type of documentation system
      - flatbed scanner or laser scanner
    - Software analysis package
  - Its ease of operation
  - Is further analysis as to protein identity or sequence desired or necessary
    - Should mass spectrometry be considered

# Vaccine Potency Testing

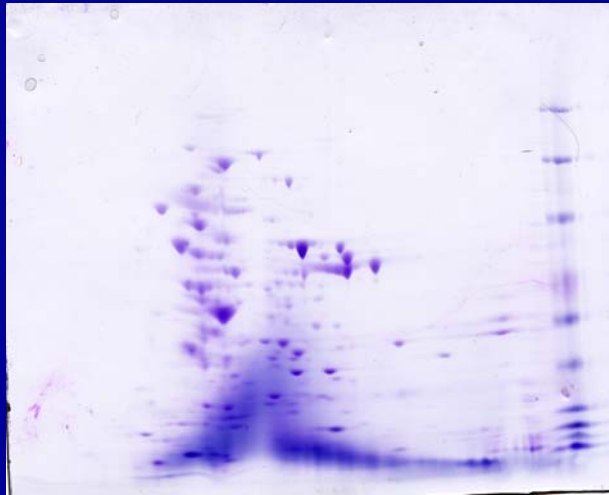
- 9CFR Part 113 January 1, 2002
  - (3) Establishing satisfactory potency for the product in accordance with the following provisions:
    - (i) Potency for live products may be determined by log<sub>10</sub> virus titer or determining the live bacterial count based on the protective dose used in the Master Seed immunogenicity test plus an adequate overage for adverse conditions and test error; and
    - (ii) Potency for inactivated products may be determined using tests for relative antigen content by comparing the antigen content of the test serial to a reference preparation using a parallel line immunoassay or equivalent method which measures linearity, specificity, and reproducibility in a manner acceptable to APHIS

# Can proteomics provide a basis for in vitro potency testing?

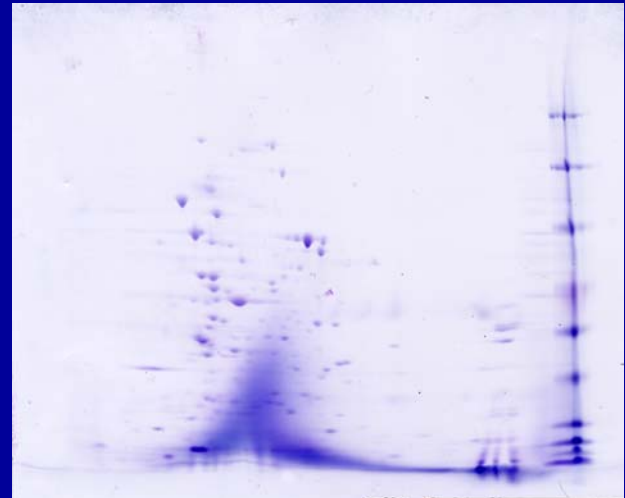
- Is proteomics 2-D gel approach
  - Feasible
    - Will it provide adequate standardization
  - Reproducible
    - What is the variability
  - Sensitive
    - Can minor changes be detected
  - Manageable with respect to cost
  - Desirable
    - Combination of approaches

# Some examples

Differences between cytosolic protein complement of  
two *Haemophilus parasuis* serovars



Serovar 1



Serovar 2

# Some examples

**Figure 2.** Sectors showing differences in the presence/absence between cellular proteins of different mycobacterial strains.

Comparisons between (A) *M. bovis* BCG Chicago, (B) *M. bovis* BCG Copenhagen, (C) *M. tuberculosis* H37Rv and (D) *M. tuberculosis* Erdman. Spots indicated by arrows were only present in virulent *M. tuberculosis* H37Rv and Erdman.

(a) Spots 2\_25 of *M. tuberculosis* H37Rv and 4\_25 of *M. tuberculosis* Erdman were identified as GDP-mannose-4,6-dehydratase (Rv1511; located in RD6).

