

# **2D gel Western blotting is useful for screening anti-HCP antibodies**

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# Outline

- ❖ Biologic drugs, recombinant proteins
- ❖ Recombinant protein examples
- ❖ Recombinant protein synthesis diagram
- ❖ Uses of 2DE
- ❖ Using 2D gel WB to characterize antibodies against Host Cell Proteins
- ❖ Key personnel, contact information

# Many cutting-edge drugs are biologics

## ❖ What is a Biologic?

Biological products are those derived from natural sources. They can be composed of sugars, proteins, nucleic acids, or complex combinations, or may be living cells and tissues. Examples: flu vaccine, artificial skin, gene therapy, and especially **recombinant therapeutic proteins**. The latter are derived from cultured cells or bacteria.

## ❖ How do biologics differ from conventional drugs?

**C**onventional drugs have known structures and may be chemically synthesized. In contrast, biologics are complex and not easily characterized. They tend to be heat sensitive and susceptible to microbial contamination.

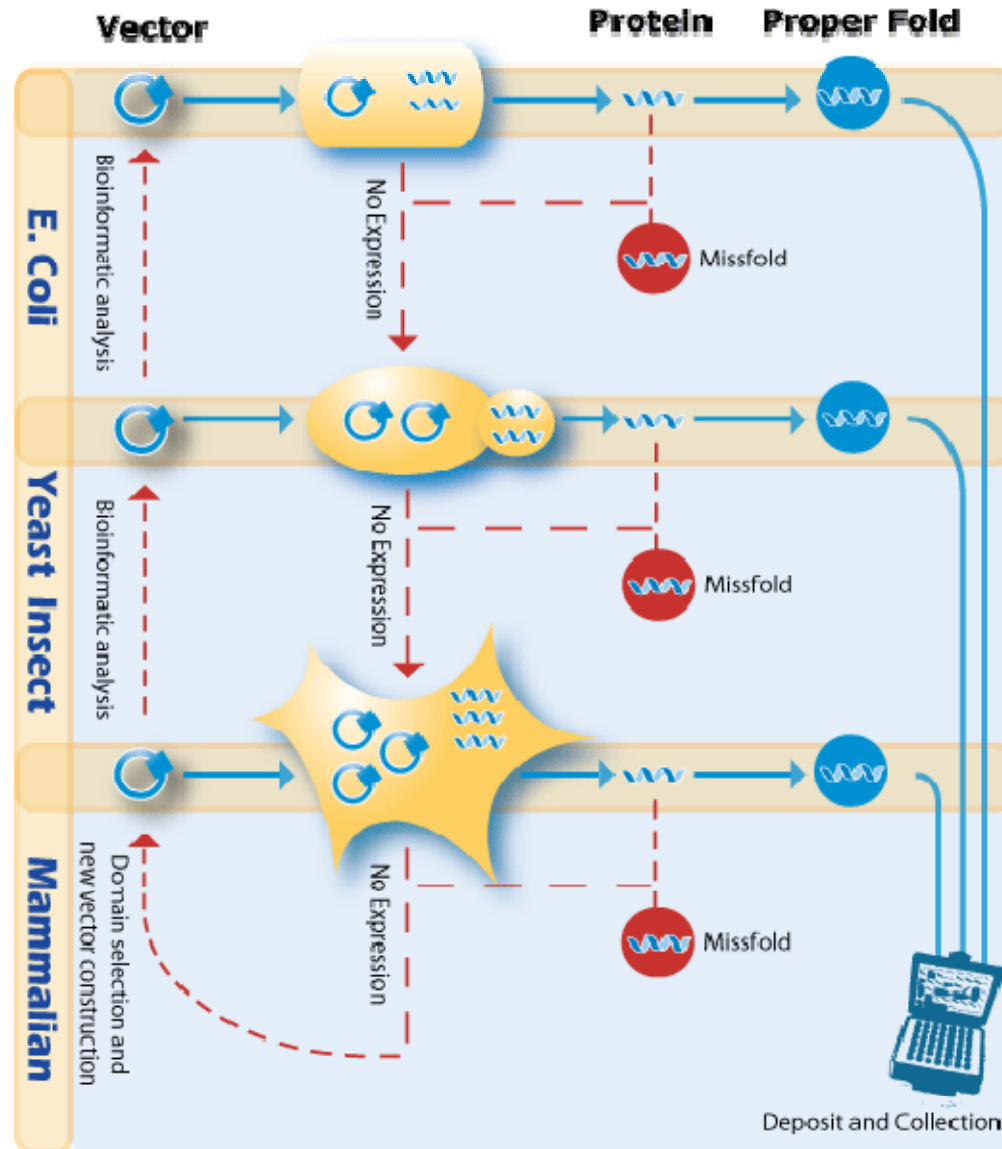
# Classes of Approved Recombinant-Protein Drugs with Examples (~100 total so far)

- ❖ **Hormones:** Insulin (Humulin, the first in 1983), human growth hormone (Humatrope),
- ❖ **Cytokines:** Interferon alfa (Roferon-A), interleukin (Proleukin)
- ❖ **Clotting factors:** Factor VII (NovoSeven), factor VIII (Kogenate)
- ❖ **Monoclonal antibodies:** anti-VEGF (Avastin), anti-EGFR (Erbix)
- ❖ **Vaccine products** Hepatitis B surface antigen (Recombivax HB),  
*B. burgdorferi* outer surface protein A (LYMERix)
- ❖ **Enzymes:** Glucocerebrosidase (Cerezyme), DNase (Pulmozyme)
- ❖ **Novel synthetic proteins:** Fusion protein of interleukin-2 and diphtheria toxin (Ontak)
- ❖ **Novel conjugates:** Covalently attached metal chelators: (Zevalin);  
Covalently attached chemotherapeutics: Mylotarg

Taken from: Dudzinski, D. & Kesselheim, A., *NEJM* **358**: 832, 2008

Recombinant Protein Synthesis Diagram  
taken from the Genway  
Biotech website:  
[www.genwaybio.com](http://www.genwaybio.com)

Host cells can be  
*E. coli*, yeast, insect,  
and mammalian  
cultured cells,  
especially CHO.

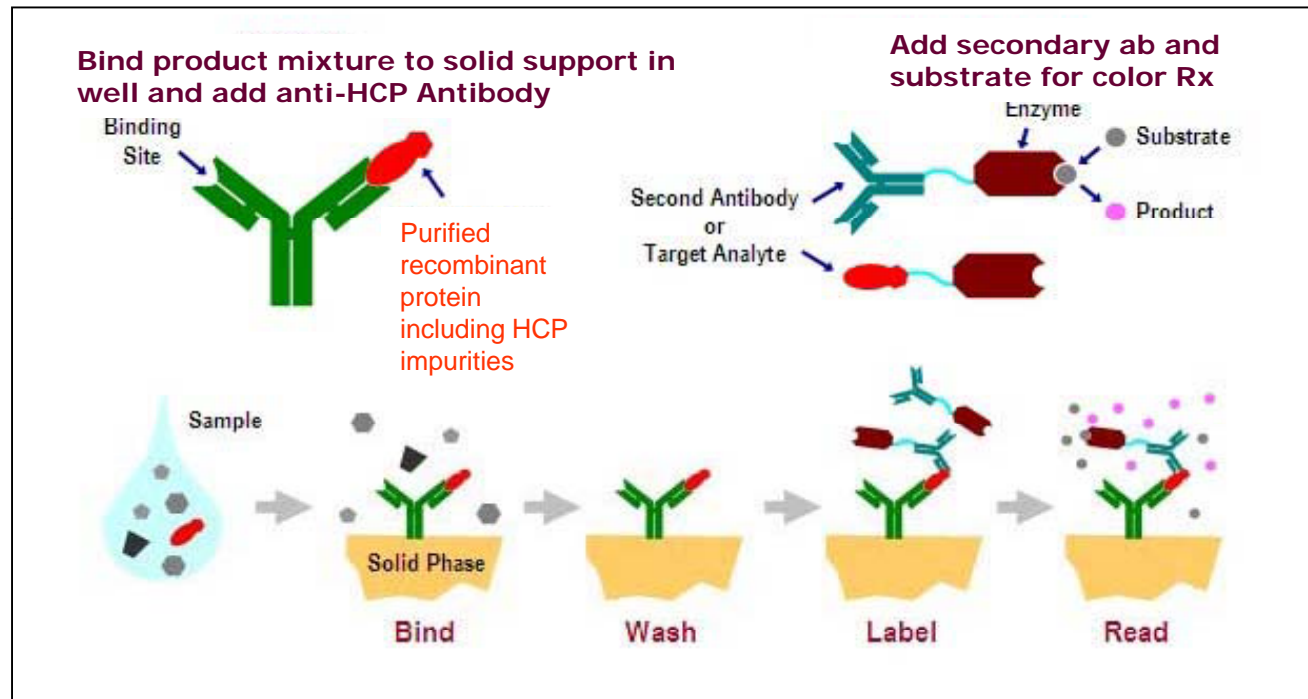


## 2D gel electrophoresis is useful at several stages of production.

- ❖ 2DE is used to characterize charge isoforms of recombinant proteins.
- ❖ 2D Western blotting (WB) with an anti-host cell protein (HCP) antibody during production steps allows identification of contaminants by MS e.g. (1)
- ❖ 2D WB may be used to characterize anti-HCP antibodies for Elisa testing.

1. Hunter, A. et. al Separation of host cell proteins oppa and dppa from recombinant apolipoprotein in an industrial hic unit operation. *Biotechnol Prog*, 2009. 25(2): p. 446-53.

To measure HCP contamination, an anti-*HCP* antibody is used to set up a quantitative Elisa assay



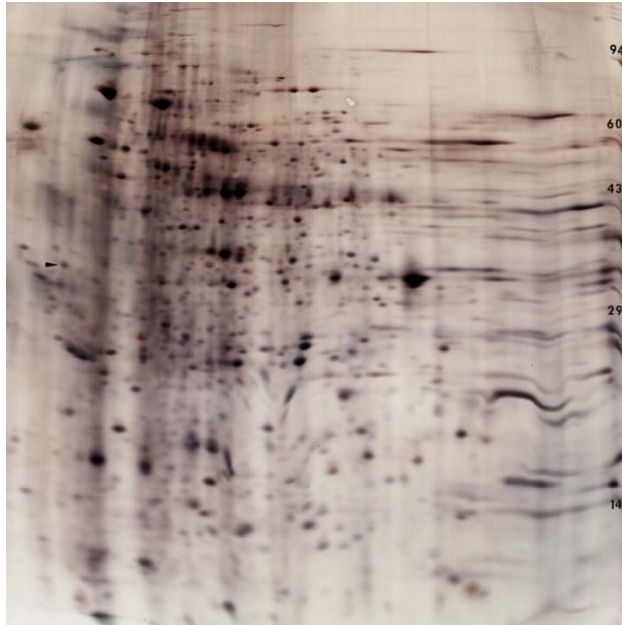
Elisa testing can be standardized and validated for GMP. However, it's only as good as the polyclonal antibody.



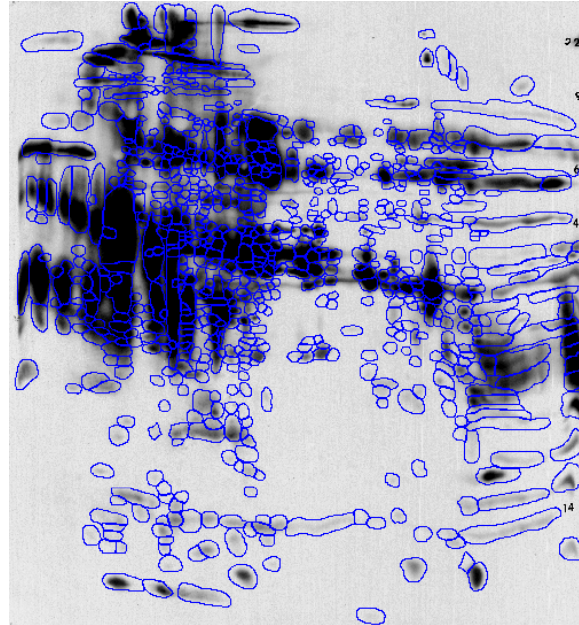
## The anti-HCP antibodies must be screened for specificity

- ❖ Kendrick Labs is getting requests to characterize anti-HCP antibodies using 2D Western blotting. The number of spots on a 2D film is compared to the number on a silver-stained pattern from the same sample.
- ❖ If the Western shows a high percentage of the silver-stained proteins, it's a good antibody.

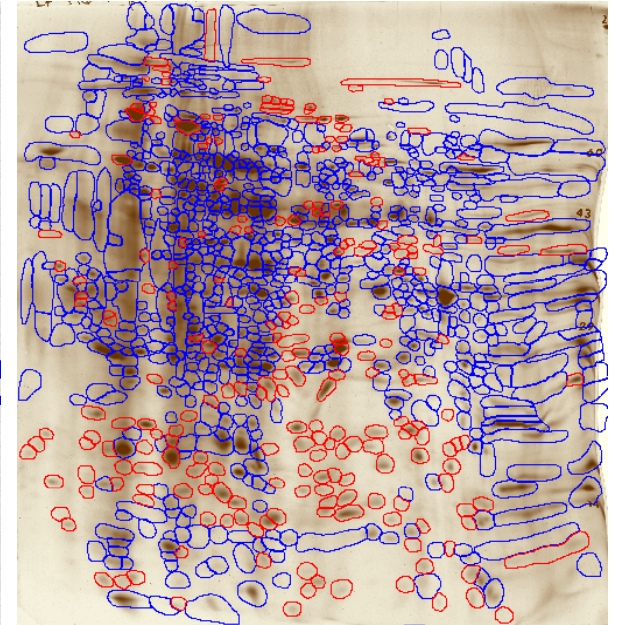
# Example of HCP Antibody Analysis



Silver-stained 2D gel from HCP shows 1045 spots with Progenesis SS



Western blot with a anti-HCP antibody shows 798 spots



Results: Red shows spots present on silver but missing from WB (blue).

Final Result:  $798/1045$  spots = 76%. Not a perfect antibody, but not bad.

## Key Personnel



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