

Host Cell Protein & Other Impurity Clearance Assays for Biosimilar Development

Omics Biowaivers, Biologics & Biosimilars- Oct 29, 2014

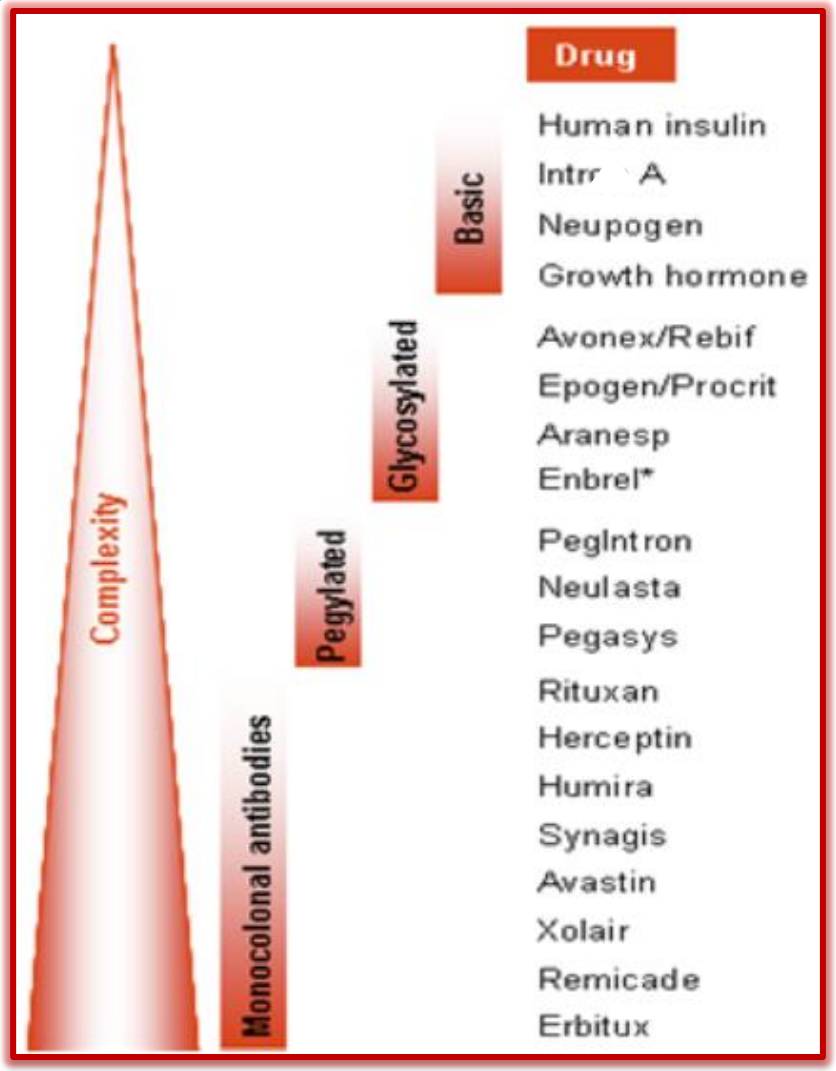
Affigenix Biosolutions Private Ltd,
265/ 1F , KSSIDC Industrial Area
Bangalore, Karnataka ,India 560099.

Company Overview-Affigenix Biosolutions Pvt Ltd



- Bangalore based CRO- Provide service in the area of TK, PK, ADA, NAb, HCP & Enzyme clearance immunoassay development.
- Registered in June 2012 & Laboratory established-May 2013
- **Facilities:** Biochemistry, Cell Biology, Microbiology~2000 sq ft
- **Experience and Expertise:** ~ > 30 years in the areas of Antibody engineering & Cell Culture, Immunology, Bioanalytical & Bioassay method development.

Experience and Expertise of the Team



Host- Recombinant Production of biologics

- Mammalian cells-CHO
- Yeast- *Pichia and Saccharomyces*
- Bacteria- *E.coli*
- Fungus- Aspergillus
- (Plant- Tobacco)

Bioprocess Development

- Protein A / G
- Enzymes- Trypsin, Chymotrypsin Catalase, Carboxypeptidase.
- Lectins- Concavalin A
- Inhibitors- Peptide Enzyme Inhibitors
- Proteins-Albumin

Press Release- Clinical Hold

FDA Puts Clinical Hold on Inspiration's Trials of rFIX August 1, 2012

"Inspiration had reported to the FDA that, during the course of routine laboratory evaluations conducted as part of an ongoing phase III clinical trial, it had discovered that a ***higher proportion of individuals treated with IB1001 had developed antibodies to proteins from the Chinese hamster ovary (CHO), than was expected*** *based on earlier study data". www.hemophilia.org*

FDA Lifts Clinical Hold on Cangene's Hemophilia Compound IB1001 – Press release- July 29, 2013

IB1001 is an intravenous recombinant FIX (rFIX) product being developed for the treatment and prevention of bleeding in individuals with hemophilia B. In February 2013, Cangene acquired all rights to the development of the investigational hemophilia compound IB1001 from Ipsen and Inspiration Biopharmaceuticals Inc. At the time of the acquisition, the asset was under regulatory review by the FDA and EMA, and ***IB1001 clinical studies were on clinical hold due to a higher than expected rate of host cell antibody development in people treated with IB1001****. Since then, manufacturing process changes have been implemented for the drug substance leading to significant reduction in the levels of HCP and comparability data confirms that the process changes have been successful. www.drugs.com*

Guidelines for Impurity Assays

- USP GC <1132> Residual HCP measurements in Biophamaceuticals. Thanks to Dr. Ranjan Chakrabarti,USP-India
- ICH Q2 (R1) –Testing impurities can be quantitative or limit test
- ICH Q6B- Clearance studies
- Guidelines on similar biologics :Regulatory requirements for marketing authorization in India-2012

7.3 Immune Responses in Animals:*Antibody response to the similar biologic should be compared to that generated by the reference biologic in suitable animal model. The test serum samples should be tested for reaction to host cell proteins.*

Host Cell Proteins (HCPs)

- **Proteome of Host Organism used in Biological productions**
  - ✓ Co-purified impurities
  - ✓ Composition and quantity vary
  - ✓ Quality attribute that need to be addressed
  - ✓ Safety Concern
  - ✓ Risk assessment: Yeast > E.Coli> Mammalian
- **Unwanted Immunogenicity**
  - ✓ Route of Injection-SC vs IV
  - ✓ Maximum dose vs Frequency of dose
  - ✓ Patient population (immunosuppressed)
  - ✓ Safety signal (preclinical & Clinical data)

- **HCP may exhibit biological activity**
- **Act as adjuvant to induce ADA response**
- **Proteolytic HCP will reduce the shelf life of the product**

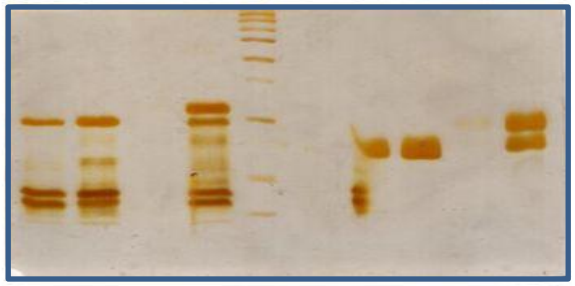
- **Clearance assay: ELISA is the gold standard**
- **Anti-HCP Immunogenicity assay (case by case)**

Process Proteins / Enzymes

Affigenix
•Devise •Develop •Deliver

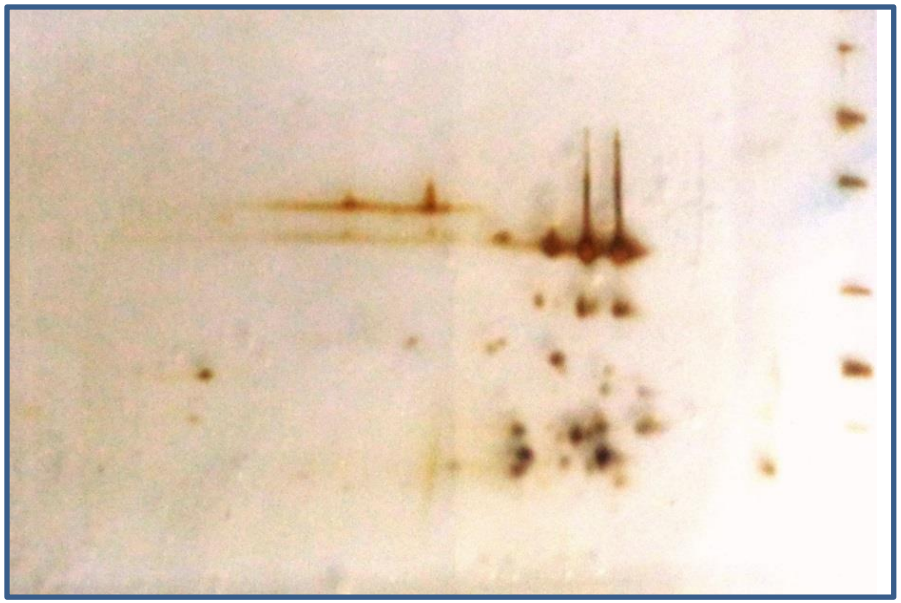
Reducing Non-reducing

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10



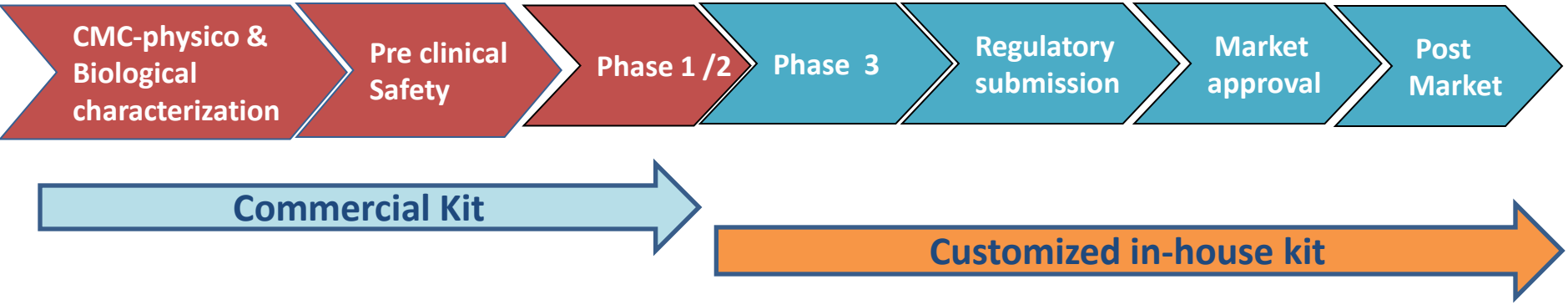
L1-Reducing bovine trypsin
L2-Reducing Porcine Trypsin -5.5µl
L3-Blank
L4-Reducing rtrypsin (expressed in pichia pastoris)
L5-Size Marker- 0.5µl
L6-Blank
L7-Non reducing bovine trypsin
L8-Non reducing Porcine Trypsin -5.5µl
L9-Blank
L10-Non Reducing rtrypsin (expressed in pichia pastoris)

Bovine Trypsin Isoforms



Validated Assay for GMP system

- In-process assay–Alert / Trend/ act
- C of A assay– Reject Limit



- Platform assay (same cell strain multiproduct assay)
- Non-transfected parental cell line.
- Mock transfected parental cell (with vector) pool
- Null cell line (vector without gene of interest)-ideal
- Upstream process specific
- Downstream process specific (enzyme used in mnf)

Host Cell Antigens / Immunogen

Source

- Same cell strain
- Null cell line or mock transfected (pool) or non-transfected
- Blank production run- small, pilot or production scale
- Supernatant, Lysates, Inclusion bodies
- Upstream process specific
- Downstream process specific.

Requirements:

- Antigen quantity enough for product life cycle.
- For immunizing the animals
- Host Goat / sheep / Rabbit / chicken
- Affinity purification
- Reference standard
- Critical reagents for capture and detection in anti-HCP immunoassay.

Customized Immunoassay Requirements

Affigenix
•Devise •Develop •Deliver

2D GE EQUIPMENTS



Ettan IPGphor 3



ImageScanner III





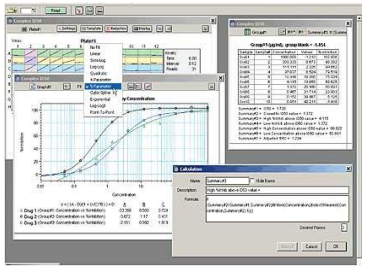
- Antibody coverage > 50 % in 2D western (linear epitope).
- Conformational coverage- depletion assay
- Assess coverage in dilution

ELISA EQUIPMENTS









- Assay must be sensitive- target for 1 ppm (no regulatory limit proposed)
- Assay must be specific-no cross reactivity to product
- Dilution linearity in ELISA
- Low cost, quantitative, throughput automation

Overview of the Workflow

Antigen characterization
(Null cell or EOF or Processes specific sample)

Anti HCP antibody generation & characterization

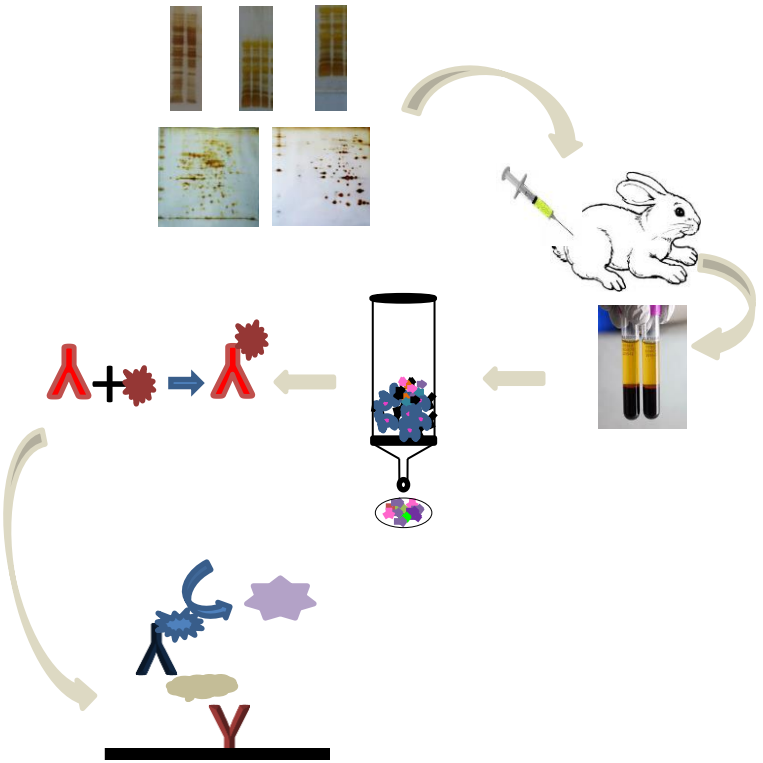
Critical reagent preparation and labelling

Qualification of Critical reagent

ELISA Method development for residual host cell protein quantification

Method qualification and validation

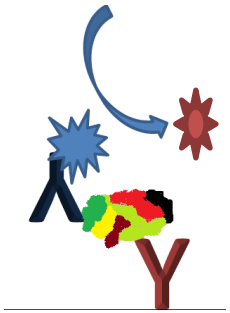
Report preparation and method transfer



HCP Assay Challenges

HCP antigen source- Null cell line, End of fermentation, also in process antigens.
Specific enzyme / protein assay used in process and its HCP
Extent of Characterization: diversity of antigens , antibody antigen complex –qualitative data requirement. Antibody coverage –demonstrate by 2 D gel.

Assay format: Bridge with polyclonal Ab

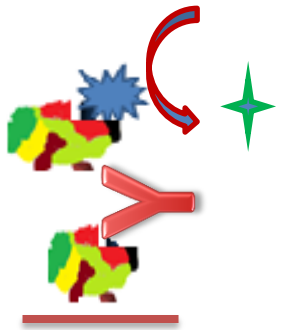


Validated assay: single product and multiproduct, process specific
Use process matrices also in the validation.
Bioprocess : Any recombinant enzyme used for bioprocess- ensure characterization of the enzyme co-purified HCP contaminants are taken into account.

Anti-HCP Assay Challenges

Biosimilar Host difference: *Pichia vs E.coli , Pichia Vs Saccharomyces sp.*

Matrix : One method for Biosimilar & Innovator



Assay format: Bridge with HCP's

Antigen source: Null cell line & product specific; scale of production

Validated assay: Single product and multiproduct

Application: Blinded or unblinded analysis

Linked Safety studies: Cytokine, Immunogenicity IgE assay, assays for other process related impurities.

Thanks to the Team Affigenix.



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THANK YOU