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# Impurities in Biotechnology Products – Experience of Setting Specifications

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# Scope of Presentation

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- Review the impurities specifically related to biotechnology products
- Experience with setting specifications for two particular impurities
- Reference to the regulatory agencies requirements relative to guidelines

# Issues Related to Impurities for Biotechnology Products

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- Complex Products
  - Many possible types of product variants/degradation products
  - Range of purity tests required to determine profile effectively
  
- Complex Processes
  - Wide variety of impurities
  - Many of these complex in nature

- ICH Q6B (1999) Note for Guidance on specifications: test procedures and acceptance criteria for biotechnological/biological products  
Chaper 4.1.3: Purity and impurities

The impurities observed in drug substances are classified as process-related and product-related:

Process-related impurities in the drug substance may include cell culture media, host cell proteins, DNA, monoclonal antibodies or chromatographic media used in purification, solvents and buffer components. These impurities should be minimised by use of appropriate well controlled manufacturing processes.

For the impurities, the choice and optimisation of analytical procedures should focus on the separation of the desired product and product-related substances from impurities. Individual and/or collective **acceptance criteria for impurities should be set, as appropriate.**

# Process Related Impurities

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- Cell culture components
  - Chemicals
  - Biologics
- Purification
  - Chemicals
  - Matrices
- Product
  - Excipients
  - Containers

# Assay of Impurities/Contaminants

Type	Target Limit	Assay Method
<u>Proteins:</u> Cell Culture Purification Host Cell DNA Microorganisms Viruses Other Process Components Product Related	ng/mg Product ng/mg Product ng/mg Product pg/mg Product ( $\leq$ pg/dose) Not Detected <Maximum Dose Endotoxin ( $\leq$ 1EU/ml) Not detected  As required  $\mu$ g/mg (0.1-1.0% w/w)	Immunoassay Immunoassay Immunoassay Hybridisation/ immunoassay/PCR Growth Tests Endotoxin LAL Test  General (Rtase/Electron Microscopy) Specific Various  Electrophoresis/HPLC

# Development of Host Cell Protein Assays

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- Regulatory guidelines EUR. PH 1997
  - Host cell derived proteins are detected by immunochemical methods
  - Antisera are raised against a preparation of antigens derived from the host organism....that lacks the specific gene coding for the product
  - This host cell is cultured and proteins extracted in the manufacturing process
  - Partly purified preparations of antigens, using some of the purification steps in the manufacturing process may also be used for the preparation of antisera

# Strategy for Host Cell Protein Testing

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- Regulatory guidelines – CPMP position 1997 (BWP/382/97)
  - `.....it is currently required that HCP be routinely monitored at the purified bulk level, using suitable analytical assays. Results from batch to batch should be consistent and meet specification limits.'
  - `.....it is impossible to set a common limit of HCP contamination for all biotechnology products.'
  - `.....standardisation of the analytical methods would be problematic as the reagents used in the tests are product and production system-related.'

# Development of Host Cell Protein Assays

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- Construct gene minus (blank) cell line
- Perform cell culture by process equivalent to manufacturing for product cell line
- Isolate cellular protein free fraction and characterise
- Raise antisera to complete cellular protein fraction
- Improve range and specificity of antisera
  - Relative to process
- Develop HCP assay

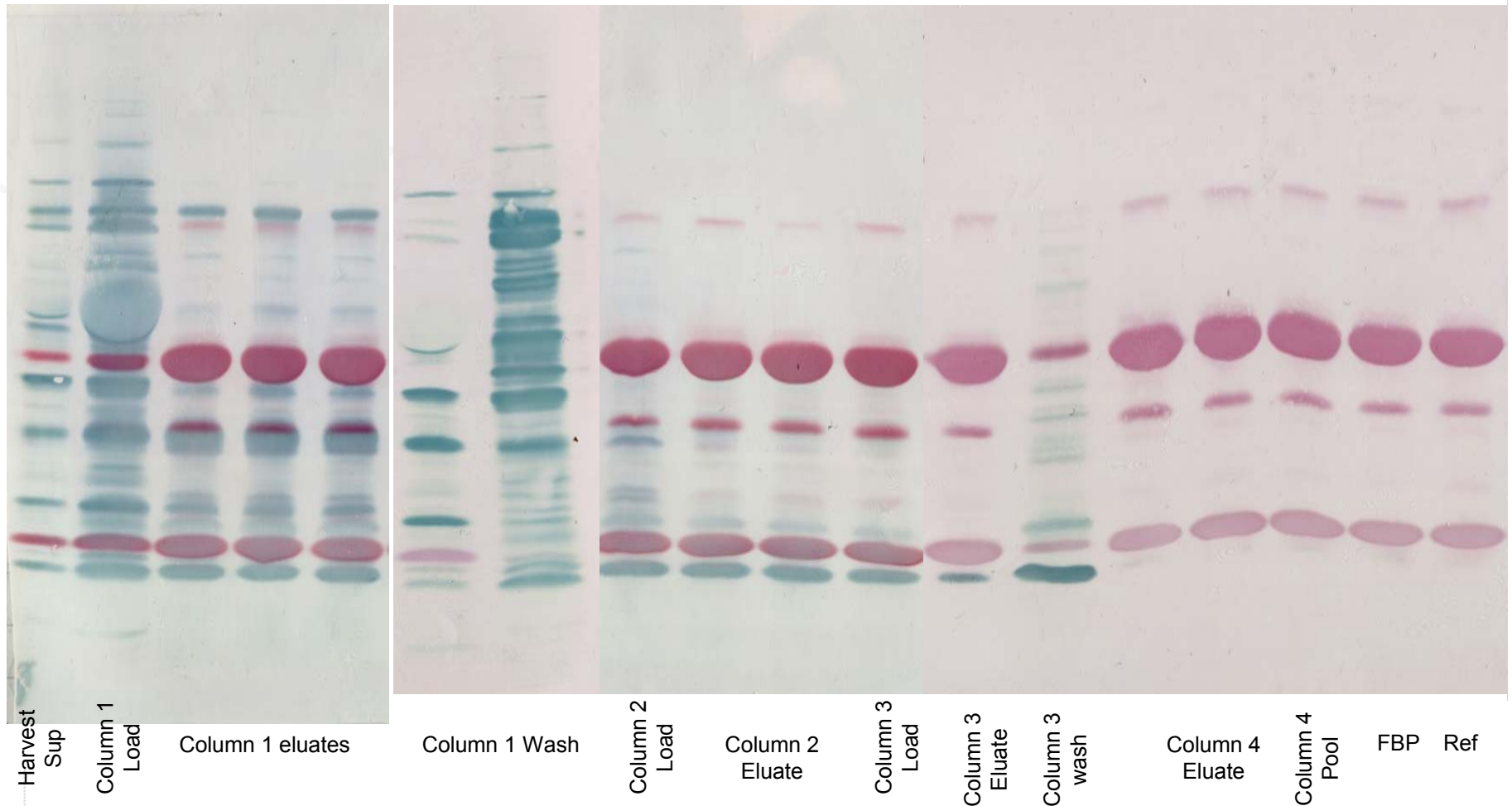
# Assay Formats for Host Cell Proteins

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- Immunoassay
  - Elisa
  - Western Blot
  
- Non-Immunoassay
  - LC-MS
  
- Combination
  - Immunocapture – LC/MS

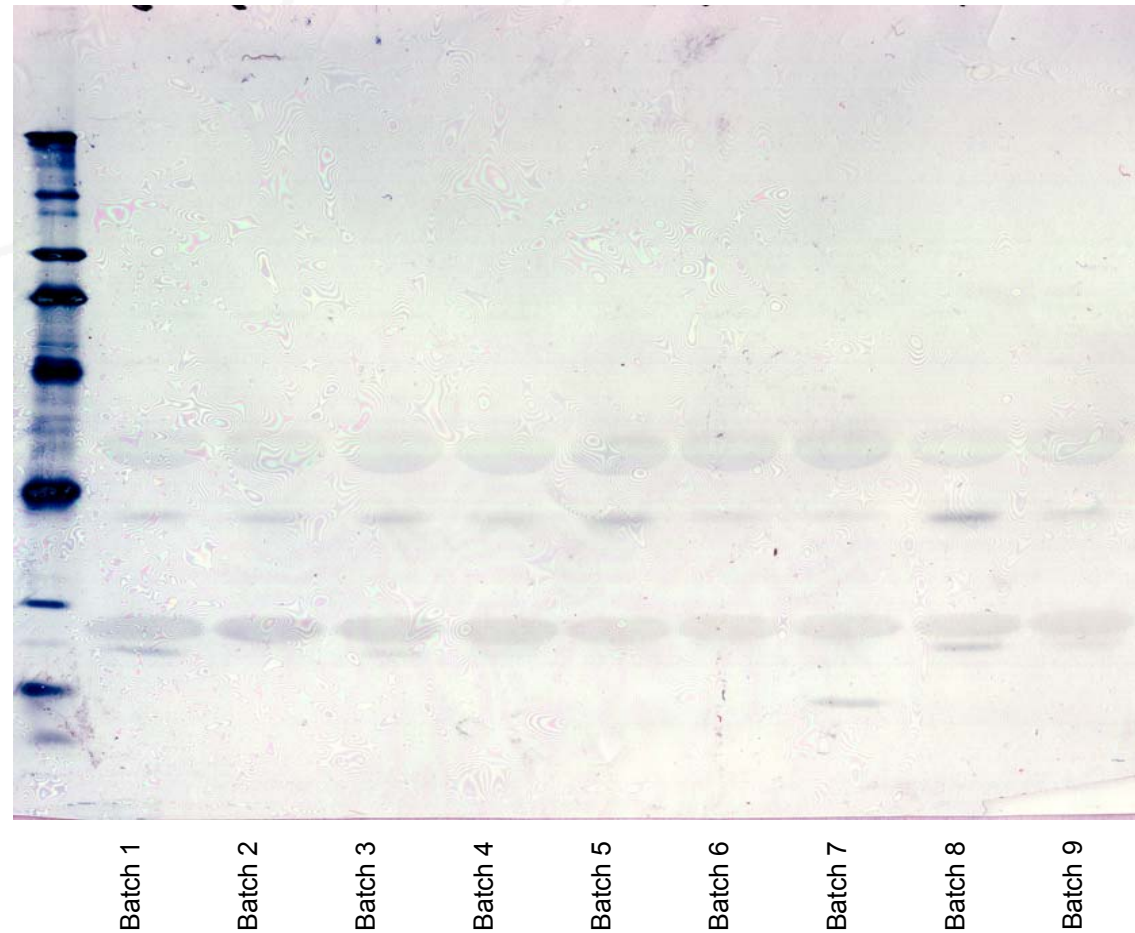
# HCP Profile by Western Blot of a Purification Process

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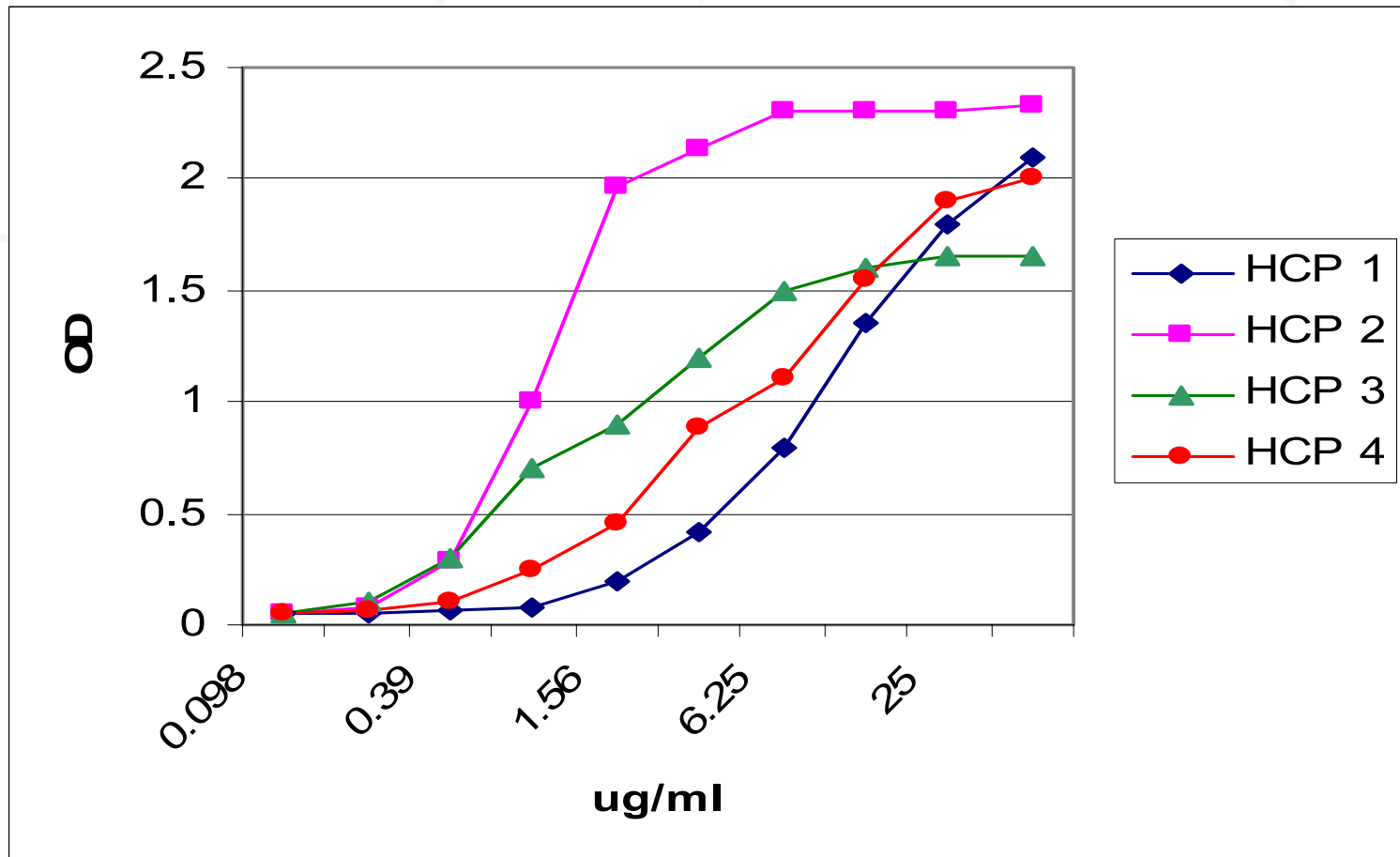


# Consistency Testing using Western Blot for HCP Impurities

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# Host Cell Protein ELISA Curves



# Host Cell Protein Specification Limits - Factors

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- Factors that may influence specification limit
  - Seriousness of disease/type of indication
  - Product dose
  - Regulatory trends

# Host Cell Protein Specification Limits - Indication

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- Classify seriousness of condition on scale 1 to 5:
  1. - Most serious
    - Acute life threatening
    - No alternative therapy
  5. - Least serious
    - Chronic condition not life threatening
    - Alternative therapy
  
- Defined dose in three categories
  - Low (L)                    - less than 1mg
  - Medium (M)                - 1mg to 100mg
  - High (H)                    - 100mg to g

# Host Cell Protein Specification Limit - Date

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- Defined in terms of date of submission:
  - Previous (P) pre-2000
  - Recent (R) 2000 or later

# Host Cell Protein Specification Limit - Experience



Condition	Approx. Spec. Range	Dose	Date	Method
1	No specific limit	H	R	No specific
1	100ppm	H	R	ELISA
2	1000ppm	H	P	Western
2	1000ppm	M	P	Western
3	1000ppm	M	P	Western
3	1000ppm	M	P	Western
4	10ppm	H	R	ELISA/Western

# Assay Format Comparison

Format	Quantitative	Identification	Limit of Detection	Use
Elisa	Limited	No	1-100ppm	Lot release testing
Western	Semi	Yes	20-200ppm (per HCP)	Process characterisation

# Host Cell Proteins Specification Limits - Summary

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- Experience of requirements for market approve
  - Change with time
  - Related to indication
  - Related to dose
  - Source/identity of host cell protein

## **CPMP (1997) Position statement on DNA routine testing versus validation studies**

A validation approach:- to validate the production process to establish that, at given steps of the purification scheme, those impurities are removed in a consistent and reproducible manner to **an acceptable level**

A routine approach:- to develop analytical tools that allow monitoring, as closely as possible, of the level of those impurities at various steps of the process and set fixed limits to be met, so that the impurities are well monitored in the final product

The validation approach:- appears to be an acceptable way, in most cases, to approach the question of residual host cell DNA



## **FDA (1997) Points to consider in the Manufacturing and Testing of Monoclonal Antibody Products for human use**

Lot-to-lot testing for DNA content prior to any excipient addition, is recommended as a way to monitor purification efficiency and reproducibility. DNA content in the final product should be as low as possible, as determined by a highly sensitive method. It is suggested that, whenever possible, the final product contain **no more than 100 pg cellular DNA per dose**. It is suggested that a method with a sensitivity of 10 pg be used to determine DNA levels.

→ **<100 pg host-cell DNA per dose**

## **WHO Guideline (1997) Requirements for use of animal cells as in vitro substrates for the production of biologicals. Requirements for biological substances No. 50:**

Additional relevant data published recently have shown that milligram amounts of human tumour cell DNA containing an activated oncogene have not caused tumours in non-human primates during an evaluation period of 10 years [1]

Based on current state of knowledge of the risks posed by residual CCL DNA, such DNA should be considered more of a cellular contaminant rather than a significant risk factor which requires removal to extremely low level and up to 10 nanogram of residual CCL DNA per dose of a purified product should be considered acceptable

→ < 10 ng host-cell DNA per dose

[1] Wierenga, D.E. Cogan J., Petriccaini, J.C., Administration of tumor cells chromatin to immunosuppressed and non immunosuppressed primates. Biologicals 22 p.221 (1995)



## **CPMP (2001) Position statement on the use of tumourigenic cells of human origin for the production of biological and biotechnological medicinal products.**

The WHO has concluded that levels up to 10 ng of residual host cell DNA per purified dose can now be considered acceptable. However, it has stated that instances do occur where CCL DNA is considered to pose a greater risk, e.g. where it could include infectious retroviral provirion sequences. The CPMP/BWP (biotechnology working party) considers that tumourigenic cells of human origin is another such instance

# Issues for DNA Assay Measurements

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- Measure DNA of potential oncogenic properties
  - WHO define as  $> 1\text{kb}$
- DNA heterogeneous in size
  - Main issue to measure DNA of regulatory concern
  - Other DNA still an issue
- Hybridisation, PCR and immunoassays methods of choice
  - Required specificity and limits of detection

# Relationship Between Assay and Process Validations For DNA

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- DNA of certain size measured by assay
- Process validation by determining clearance of labelled DNA includes all sizes of DNA
- Majority of DNA routinely lower size than that measured by the assay
- Demonstration of equivalent behaviour through the process important

# Comparison of DNA Process and Assay Validation

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Purification	Fold Removal of DNA	
	Assay	Clearance
Step		
Column 1	9000	10000
Column 2	>400	1000
Column 3	ND	8000

# DNA Specification Limits - Experience

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- Clinical studies specifications set in line with FDA guidelines
  - Assay detection limit  $<10$  pg
  - $\leq 100$  pg/dose
- Market authorisation
  - Recent experience to remove from specification where appropriate process validation data were available

# Product Related Impurities Specification Limits - Issues

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- Set specification based on clinical and process data
- Process variation including stability data
- Safety profile established
- Identification of particular impurity

# Product Related Impurities Specification Limits - Examples

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- Levels up to 2% (w/w) of known impurity generally acceptable
- Proteolytic fragment by SDS PAGE
- Aggregates by size exclusion HPLC
- Product variants by reverse phase HPLC

# Biotechnology Products Specifications - Summary

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- Regulatory requirements have changed over the past five years
- For some impurities (host cell proteins) requirements have become more stringent
- Other impurities (DNA) specification requirements have become more flexible
- Continues to be diversity based on properties of a particular product