La Vague

LE MAGAZINE DE LA PHARMA ET DES BIOTECHS

N° 49 | Avril 2016
Trimestriel

Spécial Bioproduction

Congrès A3P Bioproduction

Bruxelles, 24 & 25 mai

- **Validation Strategy of Viral Decontamination Methods, a quick overview**
- **Cahier Pratique : Quality by design approaches to viral safety of biopharmaceuticals**
- **Chromatographie continue : solution d'amélioration des performances de procédés et "debottlenecking" des capacités de bioproduction**

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L'édito

Par Jean-François Pollet

La Vague intended to announce the

3rd Bioproduction Congress

Driven by the success of the first two events, A3P will be delighted to welcome you to Brussels on 24 and 25 May for its 3rd International Bioproduction Congress. After Lyon in 2014 and Lausanne in 2015, it seemed a natural progression for the next congress to be hosted in the European capital, in the heart of a region where biotech expertise and biopharmaceutical industry prowess are represented in great number and recognised at an international level.

With a central and strategic position in Europe, Belgium, a small country with 30,528 km² and over 11 million inhabitants, boasts no less than 350 businesses active in the life sciences sector. These companies represent more than 30,000 direct jobs in the region, that is a two-fold increase in headcount in the space of 15 years. The majority of Belgian biotechnical companies are active in the pharmaceutical sector and they are responsible for 16% of the turnover of the biopharmaceutical industry in Europe. These companies represent close to 10% of the research and development spend in the European sector.

According to a study published in Nature in 2010, Belgium is considered one of the top 10 most innovative "pharma valleys". In addition to several major pharmaceutical players such as GSK biologicals, Genzyme, UCB, MSD, Pfizer, Baxter, Zoetis, Abbott, Janssen Pharmaceutica, Boerhinger Ingelheim, Amgen, IBA or Eurogentec, Belgium also boasts a multitude of local mid-size biotech actors, as well as start-ups ad spin-offs, all of which are extremely innovative in cutting edge sectors such as cell therapy and regenerative medicine. In order to strengthen the biopharmaceutical sector, which is one of the pillars of economic development for the country, Belgium is also fortunate to have many federations such as Essenscia that represent the entire chemical and life sciences industrial sector, or Bio.Be which unites Belgian bioindustries, as well as EuropaBio which represents the sector at European Institutional level. Other associations and clusters are equally active in facilitating and strengthening the development of the biotech industry at regional level: FlandersBio in the Flemish region, Biowin in Wallonia or even BrusselsLifeTech in the Brussels region.

One asset of the country is its 12 universities, two of which are considered to be in the top 100 worldwide for the life sciences sector. These universities are equipped with over 1000 research units bringing together nearly 25,000 researchers working in close collaboration with a high concentration of university hospitals and renowned research institutes. These universities provide a workforce qualified in biotechnology with, according to the study by "Conference Board", one of the highest productivity rates in the world. Belgium is also one of the European countries where the interconnection between academic institutions and the private industrial sector is at its strongest in terms of R&D partnerships, scientific publications, patent filings, and development of patents. The success of the sector is also linked to the presence of several incubators, innovation centres and extremely well-equipped science parks in terms of logistics and infrastructure.

Among the 100 drugs considered essential by the WHO, 5 were developed entirely in Belgian laboratories. Belgium is also particularly well known for its medical research into oncology, vaccinology, mental health, infectious diseases, immunotherapy and cell therapy. Belgium also has the highest number of medicines in clinical development per capita in the world. This represents no less than 200 clinical trials in phases I/II organised each year in a small country. It should also be noted that numerous financial incentives have been put in place to welcome new companies to the sector, such as the "patent box" or tax deductions on revenue relating to a patent, the reduction of labour costs for researchers, a lighter tax regime for expatriate personnel, tax deduction for venture capital, not to mention several public and private investment funds and "tax shelter" systems that foster business creation in innovation sectors

Finally, we should also add that the regulatory environment of the biopharmaceutical sector in Belgium is perfectly adapted and represented by competent and responsive authorities. In short, it represents an excellent setting for the third Bioproduction Congress which will be its coming of age!

Contributeurs

Ils ont participé à ce numéro

Merci aux membres du Comité A3P Bioproduction pour leur implication dans l'élaboration de ce numéro.
Vincent GRIFFOUL, Debiopharm - Patrick MAHIEUX, ABL Europe - Jean-François POLLET, Novadip
Alain RACHON, Merck - Anne RIGOULOT, Sanofi Pasteur

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Sanofi Pasteur
Rédacteur de "Validation Strategy of Viral Decontamination Methods , a quick overview"

LAURENT DUCRY
Lonza
Rédacteur de " Antibody-drug conjugate manufacturing techniques"

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LFB Biotechnologies

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Merck Group

Rédactrices du cahier pratique "Quality by design applied to viral safety of Biologicals: Case studies & Workshop Discussion summary"

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GÉRY VAN VYNCHT
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Rédacteurs de "Mass spectrometry as a powerful tool for the characterisation of monoclonal antibodies in the context of comparability studies."

VINCENT MONCHOIS
Novasep
Rédacteur de "Chromatographie Continue : Solutions d'amélioration des performances de procédé et "debottlenecking" des capacités de Bioproduction"

ALEXEI VOLOSHIN
3m
Rédacteur de " Enabling Higher Post Protein A Product Purity Using Novel Chromatographic Clarification Approach "

Vous aussi, vous souhaitez participer aux prochains numéros ? Faites-nous parvenir vos propositions d'articles qui seront étudiées par le comité de lecture pour approbation. => Coordonnées des contacts page 2

Billet d'Humeur

Par José Blairon - A3P Belgique

Ne vous noyez pas dans un verre d'eau !

François Morel

Notre activité professionnelle est intense mais aussi parfois frustrante. N'est-il pas préférable de la percevoir en adulte responsable plutôt qu'en victime ? Remerciez le ciel quand vous avez le moral et faites bonne figure quand vous ne l'avez pas.

En effet, la personne la plus heureuse du monde ne nage pas en permanence dans l'euphorie. Elle a aussi ses moments de découragement, ses problèmes, ses déceptions. La différence entre une personne heureuse et une personne malheureuse ne réside pas dans la fréquence – ni même dans l'intensité – de leurs coups de "blues" mais dans la façon de les appréhender.

Sommes–nous des professionnels dans ces cas-là ? Sommes-nous capables de nous remettre en question quand on a le moral à zéro plutôt que de prendre sa déprime au sérieux ? Se torturer les méninges pour comprendre et analyser ce qui ne va pas ne fait qu'aggraver la situation sans résoudre les problèmes. Une personne parvenue à un certain degré de sérénité se réjouit d'avoir le moral au zénith mais reste aussi consciente que les sentiments positifs comme négatifs sont éphémères. Pour les gens heureux ces fluctuations sont normales. Elles font partie de la vie. Plutôt que de lutter contre ses émotions quand nous sommes en colère, déprimés, débordés ou stressés, traitons-les avec sagesse et ouverture, cela nous permettra de passer avec grâce du négatif au positif. Et, ne soyons pas effrayés quand le cafard nous atteint de temps en temps ! Un coup de spleen, ce n'est pas la mer à boire. Soyons proactifs en réagissant avant que les risques potentiels de la situation ne mènent à la crise … au burnout … ce syndrome de plus en plus fréquent chez les cadres de notre industrie sans doute aussi par manque de passion pour leur métier. Essayons de voir plus loin que le bout de notre job car le bonheur ne vient pas à ceux qui l'attendent assis les pieds dans l'eau même si ça peut être agréable en fin de journée les jours du congrès A3P à Biarritz.

Un conseil : la prochaine fois que vous aurez le moral en berne, au lieu de résister, essayez de vous détendre en prenant la situation avec élégance … hissez haut le drapeau aux couleurs de l'optimisme. Au niveau professionnel, il faut en permanence chercher l'équilibre, le juste milieu entre le souci de détail et l'efficacité, ses sensations personnelles et le travail en équipe, la productivité et l'humanité, l'enthousiasme et la prudence, la rigueur et la tolérance. Dès lors, vos sentiments négatifs s'en iront aussi sûrement que le soleil va se coucher le soir et vous serez de nouveau gai comme un pinson.

Actualités A3P

Par Dominique Sierakowski - Octapharma

Bilan de l'enquête "Bonnes pratiques de mapping thermique en lyophilisation"

Qu'entendons-nous par le "mapping thermique" dans le procédé de lyophilisation ?

G.I.C. LYOPHILISATION

Il y a :

- la mesure de température de surface des étagères vides en qualifications du lyophilisateur initiale et périodique,
- la mesure de température des produits lors de cycles de développement, de transferts industriels, de validation et de production de routine.

Ce sujet continuant à susciter de nombreuses questions, le GIC LYO a proposé à A3P de réaliser une enquête auprès des industriels pour connaître les pratiques actuelles et évaluer l'intérêt pour le sujet. Entre le 18 décembre et le 14 janvier 2016, 18 sociétés concernées ont répondu à un questionnaire.

Les résultats de l'enquête montrent une certaine disparité sur les pratiques. Les questions posées confirment un intérêt aussi bien pour les pratiques que pour le matériel et la méthodologie utilisés.

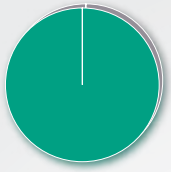
Le GIC LYO a donc décidé de coordonner un groupe de travail élargi afin d'élaborer un cahier pratique sur "bonnes pratiques de mapping thermique en lyophilisation"

Objectif : *Avoir un projet de "draft" qui sera présenté et discuté lors des prochaines Journées Lyophilisation en 2017.*

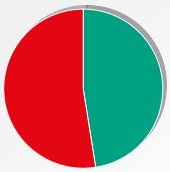
Si vous êtes intéressé(e) pour intégrer le groupe de travail vous pouvez contacter A3P quelques places sont encore disponibles.

Résultats de l'étude

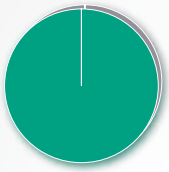
1. Sur vos installations de lyophilisation, réalisez-vous :



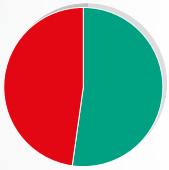
100% OUI — Une **cartographie** de température des étagères vides, en **qualification initiale**



52,4% NON — Une **cartographie** de température des étagères vides, en **qualification périodique**

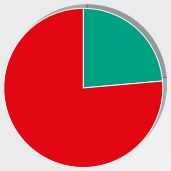


100% OUI — A la suite d'une **intervention technique sur le circuit des étagères**



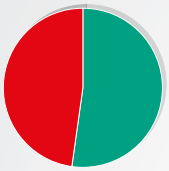
52.4% OUI — A la suite de **non-conformité** sur le produit lyophilisé

2. Sur vos procédés de lyophilisation, afin d'évaluer les transferts thermiques et connaître la dispersion de la température des produits dans la chambre de lyophilisation, réalisez-vous une cartographie de température des produits :



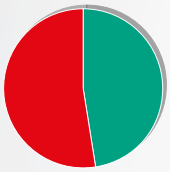
76,2%
NON

Pour **évaluer** et **déterminer** le "**design**" des articles de conditionnement



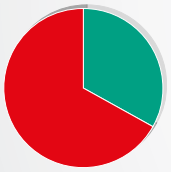
52,4%
OUI

Pour le **développement des cycles** de lyophilisation (R&D)



52,4%
NON

Pour le **transfert de technologie** d'un lyophilisateur A vers un lyophilisateur B



66,7%
NON

Pour les **lots de validation**

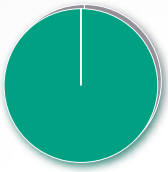
90,5%
NON

Pour les **lots de production de routine**

3. Si vous réalisez le suivi de la température au niveau des produits pour la production de routine, utilisez-vous l'information :

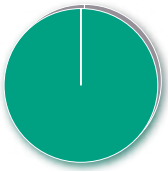
100%
NON

Pour la **conduite du cycle** (transition entre les phases)



100%
OUI

Pour **information** et/ou **vérification** de la température des produits



100%
OUI

Pour **ajuster le cycle** de lyophilisation en cas de déviations

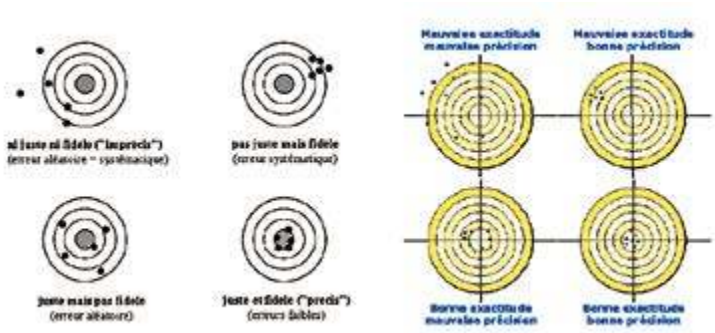
STERIDICO

Par Dominique Weill - DoW.e.l.i Sarl

C ...comme Calibre ou Etalon

Chers amis lecteurs, Calibrage ou Etalonnage or Calibration ?! Qui n'a pas redouté lors d'une quelconque rédaction d'employer un terme inadéquat voire l'orthographier incorrectement ?
Notre langue pleine de nuances, si riche reflète une capacité originale d'analyse philosophique des concepts bien française, à couper les cheveux en 5 diront certains, ce qui permet d'être logiquement plus fin, plus précis et plus juste, ce qui n'est évidemment pas synonyme !!

Bien sûr tout le monde se souvient des petits graphiques ô combien mnémotechniques démontrant que plus de précision n'implique pas plus d'exactitude.



Apporter plus de précisions ou fidélité ou répétabilité à grand renfort de détails, augmenter nombre de mesures, etc … n'apporte pas plus d'exactitude dépendant elle des erreurs systématiques comme le décalage du zéro…ou aléatoires dues aux opérateurs…

Une montre-chronomètre précise au 100ème de seconde peut ne pas donner l'heure exacte. Aussi cherchons à être précis et exact à propos de calibrage, c'est-à-dire dans notre domaine, l'action de calibrer ou opération d'ajustage (réglage) des valeurs généralement pertinentes ou critiques du procédé, par un opérateur qualifié (mais non obligatoirement certifié par un organisme officiel certifié), des écarts relevés entre les valeurs des mesures fournies par l'appareil et celles provenant d'un instrument étalon, dans les mêmes conditions opérationnelles.

Les puristes préfèreront peut-être la définition équivalente du V.I.M. (Vocabulaire International de Métrologie) celle de l' Ajustage :

Ensemble d'opérations réalisées sur un système de mesure pour qu'il fournisse des indications prescrites correspondant à des valeurs données des grandeurs à mesurer

NOTE 1 : Divers types d'ajustage d'un système de mesure sont le réglage de zéro, le réglage de décalage, le réglage d'étendue (appelé aussi réglage de gain).

NOTE 2 : Il convient de ne pas confondre l'ajustage d'un système de mesure avec son étalonnage, qui est un préalable à l'ajustage.

NOTE 3 : Après un ajustage d'un système de mesure, le système demande généralement à être ré-étalonné.

Pour information le terme "calibration" est un anglicisme pur provenant de "calibration" dont la traduction est "étalonnage". Toutefois en aucun cas, comme cela s'entend parfois et même si c'est compréhensible, la calibration n'est pas l'ensemble des opérations de calibrage. Cette confusion est entretenue par la phonétique et la littérature étrangère car au moins une vingtaine de langues utilise la racine Kalibr… pour l'étalonnage.

Quant à l'étalonnage d'instrument de mesure bien sûr, pas le dressage de chevaux reproducteurs, sa définition ci-dessous (V.I.M.2008) exclut tout réglage ou ajustage et se limite à établir une relation c'est-à-dire connaître la valeur des écarts entre les valeurs produites par l'étalon et celles de l'instrument comparé :

Opération qui, dans des conditions spécifiées, établit en une première étape une relation entre les valeurs et les incertitudes de mesure associées qui sont fournies par des étalons et les indications correspondantes avec les incertitudes associées, puis utilise en une seconde étape cette information pour établir une relation permettant d'obtenir un résultat de mesure à partir d'une indication.

Sur le plan normatif l'ISO 11139, dont la révision 2016 attendue pour la fin d'année, s'est contentée de reprendre à l'identique la définition ci-dessus mais sans évoquer le calibrage ou ajustage. Quelques échanges avec d'autres experts m'ont laissé apprécier que le monde anglosaxon n'est que peu sensible à la différence entre ces termes et donc n'a pas ressenti l'utilité de créer un terme spécifique dans la langue de Shakespeare.

Demeure l'ambigüité sur laquelle le consensus n'est pas de mise et les textes ne se prononcent pas : si assurément la délivrance de certificat d'étalonnage requiert le savoir faire et la maîtrise des méthodes d'étalonnage par un personnel qualifié appartenant à un organisme certifié, qui peut pratiquer l'opération d'étalonnage ? Il semble bien que seules des **vérifications d'étalonnage** et des **calibrages** ou **contrôle de calibrage** peuvent être réalisées par tout service de métrologie formé mais non certifié.

Mais alors Michel Audiard qu'en pensez vous ?
Oh, dans les situations critiques, quand on parle avec un calibre bien en pogne, personne ne conteste plus. Y'a des statistiques là-dessus. Quant à la bêtise elle ne se mesure pas au mètre-étalon puisqu'on n'en voit pas à Sèvres.

Amis lecteurs, cette rubrique n'a d'autre ambition que de vous servir.
Si vous souhaitez réagir, enrichir, participer : contribuez au SteriDico

DoW.e.l.i Sarl : d.weill@doweli.fr

Actualités A3P

Par José Blairon - A3P Belgique

21 avril 2016

23ème Forum A3P BELGIQUE

"Equipement Cleaning : Good Practices and Validation".

Un nettoyage approprié des équipements de production est essentiel dans le but de réduire les risques de cross-contamination, d'éliminer les résidus éventuels pouvant se retrouver dans le produit fini et de limiter la charge microbienne avant stérilisation ou utilisation directe.
Cette opération fait d'ailleurs l'objet de directives appropriées reconnues par tout le secteur pharmaceutique.
De nombreux textes en relation avec le nettoyage des équipement viennent d'être ajoutés dans les textes réglementaires, que ce soit dans l'Eudralex (Volume 4 Chapitre 5.20, Annexe 15), dans les PICs (Guide to manufacturing practice for medicinal products – Annexe 15), au niveau de l'EMEA (Health based exposure limits) et au niveau de l'ICH M7 (Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk). Les procédures de nettoyage des équipements ne peuvent évidemment être appliquées que si elles ont été validées sur base d'une analyse de risque tenant compte entre autres de l'utilisation des équipements pour des productions "multi produits/solutions" et de la classe des matières premières mise en œuvre (excipients, principes actifs).
A l'occasion de ce forum, nous développerons principalement les aspects validation et bonnes pratiques du nettoyage des équipement de production.

Rendez-vous donc dans les environs de Wavre (au Château ferme de Profondval), le 21 avril 2016 ...
avec en fin de journée, la visite d'une installation de production aseptique chez GSK Vaccines à Wavre Nord.
Informations & inscription www.a3p.org

Actualités A3P

24 & 25 mai 2016 à Bruxelles,

Congrès A3P Bioproduction

A3P BioProduction
International congress

DIAMANT Conference & Business Center // Brussels

24-25 May 2016

The agenda has been drafted to present case studies & testimonies from users with a solid industrial experience and who can provide a valuable feedback about the gains and the pains they encountered during some recent projects execution.

The 3 topics we have selected for 2016 are:

Biotech Facility Design & Engineering / Continuous Processing / Regulatories applied to Biological's. Our audience is coming from Process Development Manufacturing, Engineering & QA/Regulatory people.

The 2 days program has been designed to provide a good balance between plenary lectures delivered by key opinion leaders, interactive workshops co-animated by a tandem of biotech users & trusted suppliers, direct interactions at the supplier's booths and the choice between plant visits.

The multidisciplinary nature of the congress as well as the unique interactive atmosphere of all the A3P events, are creating THE real differentiating factors among other existing forums. Indeed, each congress section has been though to generate fruitful exchanges between speakers, animators, users, suppliers, experts, facilitators through panel's discussions, interactive workshops, coffee breaks & lunch cocktails at the supplier booths.

2015,

156 companies // 11 countries

245 people

74% Attendees

26% Exhibitors

Inscriptions sur www.a3p.org

12 Lectures

BioProduction International congress
DIAMANT Conference & Business Center // Brussels
24-25 May 2016

Bioprocess Technologies : Evolutions Towards 2020
Alain BERNARD, **UCB**

User Requirement Specification to Virtual Reality Platform for Designing a Biomanufacturing Process
Massimo NOBILE, **BIOARK**

Adressing Industrial Challenges Through Flexible & Cost Effective Plasma Facility Design
Geoffrey POT, **BAXALTA**

Improved Conventional Practices of the Pharma Industry Serving Cost of Goods Reduction for Autologous Cell Therapy. One Case Study : an Autonomous Tailor Made Isolator
Philippe WILLEMSEN, **PROMETHERA BIOSCIENCES** & *Claude DEDRY,* **PHARMA TAILOR EQUIPMENT**

Closing the Gap : Evaluation of New Single Use Technologies for Fermentation Process
Marjorie MONNET, **SANOFI PASTEUR**

Continuous Processing in the Past, Today and in the Futur
Andreas GLÖCKNER, **RENTSCHLER**

Sterile Ready to Use Single Use Ultra Filtration System Implementation in a Vaccine Production
Patrick EVRARD, **GSK VACCINES**

Regulatory
OMS

PAT for Bioproduction Monitoring and Control - Case Study of Inline and Automated Cell Counting
Philip MATHUIS, **OVIZIO**

Statistical Process Control Supporting Analytical Method Robustness
Meriem ABDENNOUR, **SANOFI PASTEUR**

Continuous Chromatography for Biomolecules: Process design, Process control & Regulatory considerations
Vincent MONCHOIS, **NOVASEP**

Belgium moving ahead in Biomanufacturing trends of the Future
Annie VAN BROEKHOVEN, **AMATSIQBIOLOGICALS** & *Patrick STRAGIER,* **BIOWIN**

3 Plant Visits

BioProduction International congress
DIAMANT Conference & Business Center // Brussels
24-25 May 2016

GSK (Wavre), Masthercell (Gosselies) or Zoetis (Louvain-La-Neuve)

Inscriptions sur www.a3p.org

10 Workshops

BioProduction International congress
DIAMANT Conference & Business Center // Brussels
24-25 May 2016

W.1 # Toward Continuous Bioprocessing: Industry Leader and Supplier Perspectives for the Development of a New, Integrated Mab Purification template

Gorazd HRIBAR, **SANDOZ BIOPHARMACEUTICALS &** *Frederic SENGLER,* **MERCK LIFESCIENCE**

W.2 # Built Your Own Culture Monitoring Device Today !

Sören WERNER, **ZURICH UNIVERSITY &** *Gernot JOHN,* **PRESENS**

W.3 # Biosimilar Development: Turning relevant R&D biosimilarity assessment into safe and consistent batch production

Fiona GREER, **SGS LIFE SERVICES &** *Alex KUDRIN,* **BIOPHARMA CONSULTANT / Ex MHRA**

W.4 # Comparison of stainless steel vs. single use upstream microbial biomanufacturing

Juozas SIURKUS, **FERMENTAS BIOMANUFACTURING CENTER &** *Camille DESROUSSEAUX,* **THERMO FISHER SCIENTIFIC**

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Actualités A3P

Détection des mycoplasmes, contrôle des impuretés & transfert de site industriel.

A3P organise le **16 juin 2016**, une journée scientifique "un Jour, un Labo" autour des thèmes "Détection des Mycoplasmes et Contrôle des Impuretés", avec 3 temps forts : des conférences, le retour d'expérience d'un transfert de site et la visite du nouveau laboratoire SGS.

Cet événement axé sur une thématique permet d'associer l'actualité réglementaire, l'expertise scientifique et le retour d'expérience d'industriels aux réalités opérationnelles de sites de R&D et de production.

Des conférences données par des représentants de l'industrie pharmaceutique permettront aux participants de comparer les pratiques et répondre à leurs questions:

- Comment éviter les risques d'une contamination par des mycoplasmes ?
- Quelles solutions analytiques et méthodes alternatives ?
- Quels enjeux et perspectives réglementaires ?
- Comment contrôler les impuretés élémentaires dans le cadre de la mise en œuvre de l'implémentation ICH Q3D ?
- Quelle stratégie de transfert d'un site industriel et qualification ?

Cette première édition se déroulera à Villeneuve-La-Garenne (92).
La journée s'achèvera par la visite du nouveau laboratoire d'analyse pharmaceutique SGS.

Informations & inscription sur www.a3p.org

Validation Strategy of Viral Decontamination Methods, a quick overview.

By Dr. Lionel GERENTES, PhD - Sanofi Pasteur

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Even though much progression has occurred in terms of quality and compliance in the biopharmaceutical environment, the industry is still mired in vague definitions of "decontamination" very often leading to amalgams with the actions of "cleaning" and "disinfection". This confusion, particularly in the bio-pharmaceutical realm of infectious antigens and particularly viruses, leads to situations of non-technical mastery, non-compliance and ultimately increased risk of cross contamination between products. All of which stress the importance of clarity in biocontainment.

In the early 2000's, due to external pressures, the industry was obliged to remove formalin (CMR) which was widely used as a decontamination reagent. Adopting alternative decontamination reagents highlighted three main points: (i) historical decontamination exposed the relative ineffectiveness of formalin in the light of current practices; (ii) validating the performance efficiency of decontaminants is difficult (too many external variable factors influencing its performance); (iii) highlighted the need for in depth performance review of all the tools and decontamination processes. These observations are further supported by Polio eradication ambitions conducted by WHO (GAP-III) which also highlighted these gaps and weaknesses.
Nowadays, associated technologies of different decontamination methods (i.e., Physics, Thermal, Chemical) are numerous, and provide a wide panel of choices that can be employed in the biopharmaceutical industries, and especially vaccines companies. Accordingly, the duty of mastery and performance validation of decontamination processes is no longer an option (!) However, new constraints are emerging and require substantial human and technical resources impacting the project costs, perhaps exponentially running into millions of euros!

This article aims to serve as a "lessons learned" and is on based many years of alternative decontamination investigations. The article also shares the strategy, initially conceived in 2004, that was designed to anticipate the new paradigm of state of the art of decontamination. Finally this article aims to participate in the education on this often misunderstood and often over looked topic…

Definitions

It is important to clarify the pharmaceutical definitions of "cleaning" and "disinfection", and further elucidation can be highlighted by examples.

Cleaning

Result of an operation in a limited time, allowing the withdraw of all undesirable inert compounds acquired on contaminated surfaces according to laid down objectives. The result of this operation is limited to the compounds being present at the time of the operations.

These compounds come from natural environmental sources or from the product handled.

Objectives aimed by **CLEANING** *are inert compounds (Production or laboratory's areas)*

Disinfection:

Result of an operation in a limited time, allowing the withdraw, inactivation or killing all undesirable micro-organisms carried by contaminated inert media according to laid down objectives. The result of this operation is limited to the micro-organisms being present at the time of the operations (AFNOR NFT 72-101).

These micro-organisms are not specific and come from natural environmental sources.

Objectives aimed by **DISINFECTION** *are the environmental micro-organisms (Production or laboratory's areas).*

The definition of decontamination can flow from the two previous ones:

Decontamination:

Result of an operation in a limited time, allowing inactivating, killing or destroying all specific micro-organisms handled according to laid down objectives. These micro-organisms are known and specific.

Objectives aimed by **DECONTAMINATION** *are to control of the dissemination of the specific micro-organisms (vaccine products or micro-organisms handled in laboratory)*

In conclusion, the use of the disinfection term as a synonym of decontamination has to be prohibited. Finally Cleaning does not insure disinfection or decontamination. Similarly, disinfection does not insure decontamination or cleaning.

Strategy of viral decontamination

Considering all of the decontamination technologies (physical techno's, chemical reagents…) with differing mechanisms, that we will call the "Weapons" (see tables 1 & 2) coupled with the huge number of viruses, that we will call the "Targets", the list of validations to perform may become unmanageable, lengthy and cost prohibitive.

Chemical modes	
Liquid reagents	In depth **and /or** surface decontamination
Gaseous	**Mainly** surface decontamination
Physical modes	
Radiation	In depth **and** surface decontamination
(pulsed) **Light**	In depth **and/or** surface decontamination
e-beam	(mainly) Surface decontamination
Thermal modes **(Autoclave, Oven)**	**Mainly** used for in depth decontamination

*Table 1: Decontamination modes, that we will call the "**Weapons**"*

Fortunately, viruses elicit interesting properties such as (i) Their inability to generate resistant mutation against chemical reagents (because resistant mutation occurrence can only be acquired during their viral replication which is not the case here) (ii) Their composition with 4 basic compounds, Nucleic acids, Amino acids, sugars and lipids which essentially transform viruses in to simple chemical targets rather than "daunting" viruses.

Considering these new paradigms of viral properties, possibilities emerge including a "bracketing strategy" to create virus models representing the worst case scenarios. Obviously, the rule of bracketing can't generalized absolutely, but can be linked to a clear and a strong scientific rational, a list of specific criteria's and also linked to a list of considered viruses. In the following example, 9 viruses routinely handled in a vaccine company will be analyzed (table 3).

After having identified the targets and the weapons, all the "**Constraints**" should be identified.

From the targets side:

The availability of the target (i.e.: level of concentration, fragility of microorganisms…), the availability of lab's capability for handling (bio-safety containment), the availability of the Methods of quantification: are they available, if yes what are their detection limits, their robustness (matrix viro and/or cytotoxicity)?

Modes / Reagents	Main Target(s) on viral structure
Temperature	Viral envelop, (Glyco)protein's , RNA then DNA
Acids / Bases	Viral envelop, (Glyco)protein's
Alcohols / Ether …	Viral envelop, (Glyco)protein's
Oxidants *(Cl-, O_3, H_2O_2, formalin, b-propiolactone…)*	Viral envelop, (Glyco)protein's, Nucleic Acids
Detergents *(ionic / nonionic)*	Viral envelop
UV / p-Light	Nucleic Acids, (Glyco)protein's

Table 2: Decontamination modes versus biochemical viral elements: impact on viral structure

From the Weapons side:
Are chemical reagent's compositions available? (i.e. nature and concentration of each component)? Are corresponding neutralizing reagents available? What is their Impact on methods of quantification due to cytotoxicity?
Because of the target constraints (susceptibility, level of concentration, expression systems…), one of strategies is to bracket the microorganisms to define the best model which will be able to cover a maximum number of them and will allow defining the efficient decontamination parameters. The microorganism model selected must be derived from minimally 3 main criteria (i) a Risk Analysis with the well-defined rules of bracketing. (ii)The physical availability of the potential microorganism's model including the infectious titer level compatible with the final objectives, and iii) the method of quantification used (lower detection limit, Its accuracy at low level, robustness…).
Aware of all these key elements, the efficiency specifications should be set up. Unfortunately clear and exhaustive regulatory guidance are lacking (French, European, US, International ones…) and if provided, is limited and do not cover all the cases, especially for viruses. (Table 4)
Regarding each decontamination mode, the regulatory specifications are not so clear and often derived from sterility assurance experiences, such as the famous "6 Log reduction".

Specifically for viral targets, one can find 4 log reduction of infectious titer using chemical mode, but in most of the viral cases, it is not appropriate. This leads us to the following questions what are the right specifications for (i) Surface decontamination, (ii) Liquid waste, (iii) solid waste, (iv) air? Without this guidance, a bibliography study is needed at least.
Most of the time, the efficient parameters claimed on the label for ready to use decontamination products are not appropriate because of a vast absence of methodological information like environmental conditions, scientific approach and minimal of performance requirements (i.e. 4 Log reduction linked to a norm…)

Viruses	Structural Composition: External Spikes : glycoprotein	Envelope : phospho-lipid	Core : protein	Genus : ARN	Conclusions following the "bracketing strategy" rules
Poliovirus *(Enterovirus)*	No	No	Yes	Yes	Viruses into group 1 Model represented by Poliovirus
Hepatitis A *(Enterovirus)*	No	No	Yes	Yes	
Influenza virus *(Flu)*	Yes	Yes	Yes	Yes	Viruses into group 2 Model represented by Influenza virus
Measles *(Morbilivirus)*	Yes	Yes	Yes	Yes	
Mumps virus *(Rubulavirus)*	Yes	Yes	Yes	Yes	
Rubella virus *(Rubivirus)*	Yes	Yes	Yes	Yes	
Rabies virus *(Lyssavirus)*	Yes	Yes	Yes	Yes	
Y-Fever, *(Flavivirus)*	Yes	Yes	Yes	Yes	
Dengue *(Flavivirus)*	Yes	Yes	Yes	Yes	

*Table 3: List of 9 considered viruses, that we will call the "**Targets**"*
Based on biochemical structure of each virus, here we can define 2 models, according to the following characteristics and the "constraints" which were identified in the specific rules set up

Specifications of chemical liquid decontamination			
Bactericide	**French norm**	**AFNOR NF T 72-170 and 171**	*5 Log reduction*
	European norm	**NF EN 1040**	
Sporicide	**French norm**	**AFNOR NF T 72-230 and 231**	*5 Log reduction*
Fungicide	**French norm**	**AFNOR NF T 72-200 and 201**	*4 Log reduction*
	European norm	**NF EN 1275**	
Virucide	**French norm**	**AFNOR NF T 72-180, 181 and 185**	*4 Log reduction*
	European norm	**NF EN, 14675/14476 and 13610**	
Specifications for chemical air decontamination			
Bactericide			*5 Log reduction*
Sporicide	**French norm**	**AFNOR NF T 72-281**	*3 Log reduction*
Fungicide			*4 Log reduction*
Virucide			*4 Log reduction (New! Nov.14)*

Table 4: examples of norms for specification setting up

Finally the Strategies of Validation can be summarized as such: The right weapon against the right target with the best tool box, which should be defined specifically. In any case, all (your) the specifications must be set up in regards of each specific use.

After more than 10 years' experience, our lesson learned has provided valuable positive experience. All (our) viruses are linked to their validated efficient decontamination parameters, in a compliant, coherent and robust system while realizing savings via our approach. Now each new potential decontamination reagent is easy to validate and a comprehensive update to our decontamination system for all viruses in can be performed in a few experiments. Moreover, the strategy has been vetted with regulatory authorities resulting in increased compliance with no significant observations.

The remaining challenge, is to educate auditors who are not all familiar with viruses reducing their preconceived notions of viral complexity thereby enabling full endorsement of the performance and efficiency of these approaches and data.

Antibody-Drug conjugate Manufacturing Techniques.

Par Laurent DUCRY - Lonza

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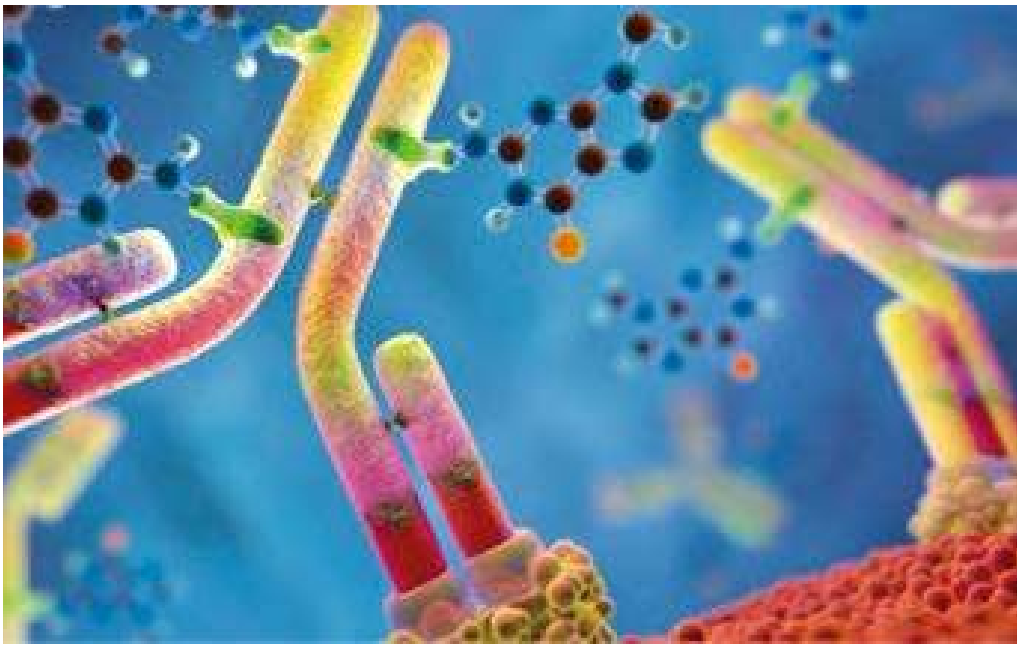

Figure1

Monoclonal Antibodies (mAbs) with selectivity towards antigens located on the surface of cancer cells have been successfully developed. Nonetheless, mAbs alone are generally not potent to kill the cancer cells and are therefore used in combination with classical chemotherapies. The small molecule cytotoxic drugs used in chemotherapies effectively kill fast dividing cells. These include but are not restricted to cancer cells, meaning that side-effects are generally associated with conventional chemotherapies. Moreover, the most potent and potentially most effective drugs cannot be used because side-effects would be too severe. An antibody-Drug Conjugate (ADC) is the unique combination of a targeting monoclonal antibody, a stable linker, and a potent cytotoxic agent[1]. It is designed to deliver anti-cancer agents directly to the tumor cell in a targeted manner to limit systemic exposure (Fig. 1).

Efforts in this field have led to the approval of two ADCs as drugs: Adcetris for the treatment of refractory Hodgkin lymphoma and anaplastic large-cell lymphoma and Kadcyla for the treatment of metastatic breast cancer. Auristatin and maytansine derivatives are used as cytotoxic agents, respectively. On average, 2 to 4 toxins are attached or conjugated to the mAb. In addition to the two commercial ADCs, close to forty are under investigation in many different cancer types.

Conventional ADCs, where conjugation takes place either on lysine side-chains or to reduced cysteines, are heterogeneous. The average drug loading as well as the subpopulation must, however, be consistent from batch to batch. The conjugation process defines the product and the average loading must be the same for each batch. In recent years, site-selective conjugation technologies have been developed. Site-selective conjugations through mAb engineering, enzymatic conjugation reactions, or more selective chemical reactions is expected to afford more homogeneous ADCs with improved therapeutic properties (Fig. 2). The current clinical pipeline is, however, still dominated by random lysine and/or cysteine conjugation, which makes analytical characterization challenging.

The conjugation reaction is followed by protein purification techniques to remove process-related contaminants (unconjugated toxin and residual solvent). During drug discovery, purification can be achieved through dialysis or with single-use Sephadex columns. These techniques are replaced

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by Tangential Flow Filtration (TFF) during process development and scale-up. Suitable conditions are developed, with the goal to achieve good impurity clearance, short TFF time and low aggregation rate. Once suitable membranes have been selected and the TFF parameters developed (feed flow, TMP, conc.), one can investigate how many diafiltration volumes are needed to clear out process-related impurities (Fig. 3). A well-developed TFF process is, in most cases, sufficient to reduce the amount of unconjugated toxin to an acceptable level. TFF, however, does not remove aggregates. If aggregate formation cannot be prevented during the conjugation process, a low-pressure chromatography purification step will be needed. In such cases, mixed-mode (CHT or MEP HyperCel) or ion-exchange resins are recommended.

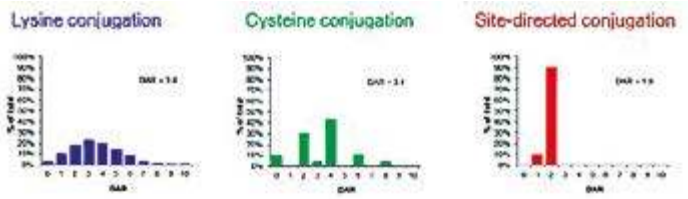

Fig. 2. Typical drug loading distribution (or drug-to-antibody ratio, DAR)

Lonza has established dedicated teams and facilities to facilitate the scale-up of ADC projects. For clinical phase 1 and 2 material, our small scale assets are generally well suited both in terms of flexibility and vessel size (10 to 60 L). For phase 3 and beyond, the process is transferred into our commercial bioconjugation plant where we have 100 to 600 L stirred tanks (Fig. 4). These volumes are small compared to antibody manufacturing, explained by the higher process concentration, shorter batch time and high potency of the API.

Some safety challenges are associated with ADC manufacturing since one has to manufacture a highly toxic compound in a biopharmaceutical

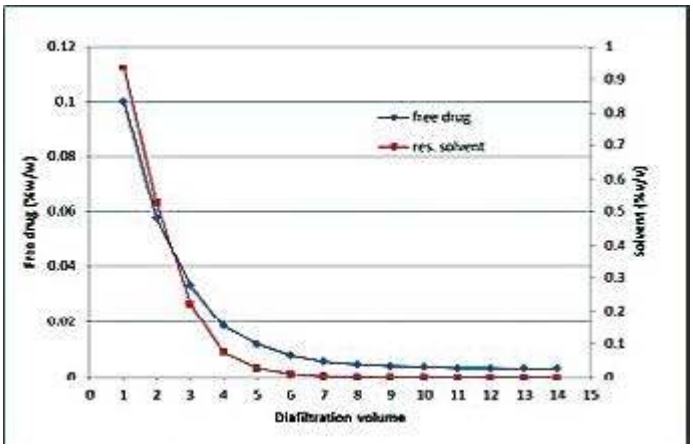

Fig. 3. Typical clearance of unconjugated toxin (free drug) and organic solvent by TFF

environment. A balance between occupational safety and cGMP requirements must be achieved. The typical ADC payloads are among the most toxic compounds ever produced and strict containment measures are needed. Typical occupational exposure levels for ADCs and their toxins are between 100 down to 1 ng/m3. But needless to say that when considering the potential benefits for cancer patients, it is gratifying to tackle the challenges associated with the ADC design, scale-up and manufacturing.

Fig. 4. Strict safety standards and procedures together with state-of-the-art equipment were specifically designed for the handling of ADCs (©Lonza Ltd)

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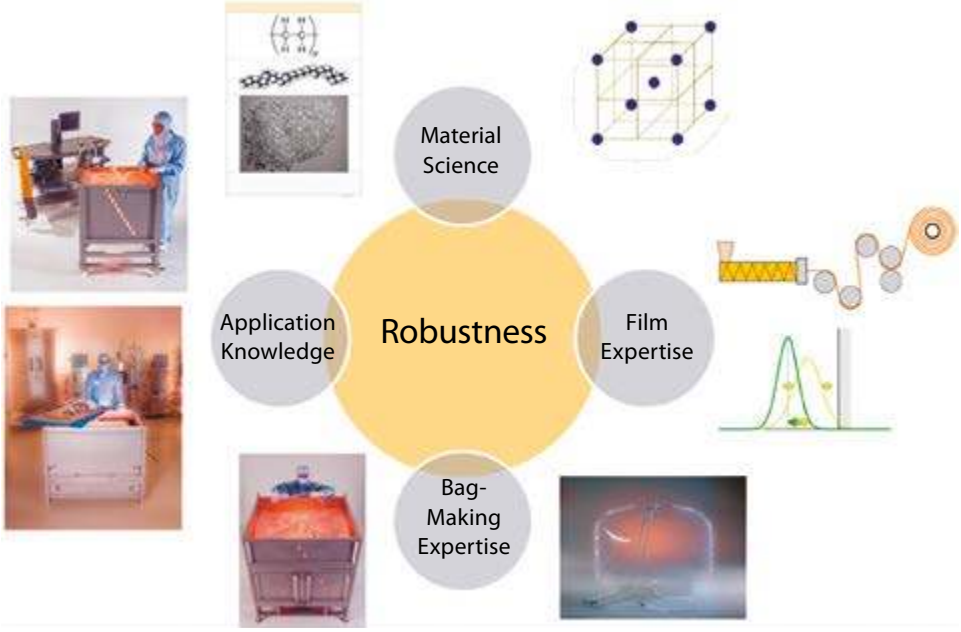
Résumé

Les conjugués anticorps-médicament, ou immunoconjugués cytotoxiques, représentent une nouvelle forme de thérapie anticancéreuse ciblée. Ils peuvent se lier à certains types de cellules cancéreuses dans lesquelles ils libèrent directement leurs agents chimiothérapeutiques.

Différents types de conjugaison ont été développés, pouvant donner une nouvelle option de traitement pour les patients atteints de cancer. Lonza résume certains aspects liés à la production et à la purification de ces composés parmi les plus difficiles à manipuler.

Robust and Convenient Single-use Processing.

By Elisabeth VACHETTE, Lucie DELAUNAY, Magali BARBAROUX, Gerhard GRELLER, Christel FENGE & Jean-Marc CAPPIA - Sartorius Stedim



The implementation of single-use addresses the main challenges of the biopharmaceutical industry, e.g. fast and straightforward capacity adaptation, cost savings, risk mitigation as no cleaning is required. Because single-use bags offer multiple technical and economic benefits[1] they are broadly adopted by the entire biopharmaceutical industry to achieve rapid and flexible development and commercial production of monoclonal antibodies, recombinant proteins and vaccines. The industry is now expanding the use of single-use bags into all bioprocessing steps and new applications such as cell and microbial culture, storage, shipping, mixing and freeze & thaw of process intermediates, and final filling[2]. With this growing implementation of single-use bags in all process steps, there are increasing requirements for more outstanding product quality, assurance of supply, change control, robustness, ease of use and scalability, throughout all applications of single-use processing, from the cell culture to shipping of bulk drug substance and final filling of drug product.

Partnership with resin and film suppliers

Developing new films and bioprocessing bags that meet all these requirements, multidisciplinary skills, knowledge and expertise are necessary. Collaboration between bag manufacturers and film or resin suppliers allows to combine material science, film extrusion knowledge with welding and bag making expertise. The specifications are set to meet the user requirements for upstream, downstream and aseptic bioprocessing. A Quality by Design (QbD) approach is used to reach a robust bag technology for all applications. This QbD approach includes the selection and specification of appropriate resins and additives, the definition of the film extrusion design space, and the validation and control of the welding process and the overall bag making process.

Quality by Design approach (QbD)

The QbD approach starts with the in depth understanding of the applications and the definition of the user requirement specification (figure 2). Today, almost all unit operations in biopharmaceutical drug development and production can be performed using single-use solutions. However, the requirements with regard to mechanical and physical properties can largely vary depending on the application. For example, a bag used in rocking motion cell culture or in liquid shipping applications requires a very flexible film resistant to material fatigue. A bag used in large scale mixing applications or in a stirred tank design has to absorb the significant hydrostatic pressure of 2000L to 3000L liquid. Here, the strength of both the film and the welds is more critical.

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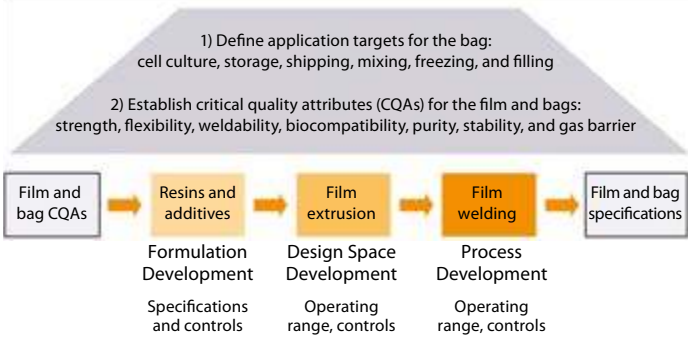

Figure 2: Quality by Design approach to ensure consistent Robustness of Flexsafe® bioprocessing bags

In depth knowledge of **material science** and **polymer expertise** are required for the selection of the right plastic resin material and the additives that will provide the required quality attributes. As a first step, theoretical robustness is designed by material science experts knowing the plastic materials and their behavior when associated with each other. The polymer experts test and select the plastic materials that confer the best film properties like the strength, flexibility, weldability, biocompatibility, purity, gas barrier and drug stability. This material selection, the number and succession of plastic layers and their thickness will then determine the final features and the overall performance of the film.

Film expertise is the second critical factor in the development of a robust single-use solution. Robustness is the result of multiple properties such as film strength, film flexibility, seal strength resistance, film weldability and material puncture or tear resistance. The in-depth knowledge of film behavior helps choosing the right plastic material mix that will achieve these properties knowing that some of them can be antagonistic. For example, a strong and stiff film is highly resistant to impact or puncture, but shows less flexibility and less resistance to fatigue. A too flexible film will not withstand hydrostatic pressure in large scale bags or positive air pressure required during cell culture or during bag integrity testing.

Film extrusion expertise is required to ensure the lot-to-lot consistency of critical quality attributes and the reproducible robustness of the film. This is achieved by defining and controlling the appropriate critical process parameters (CPP) for the extrusion process. During the development of the extrusion process, the process variability is explored via a design of experiment (DoE). The ultimate tensile strength is measured as a parameter to quantify robustness and should be consistent throughout the operating ranges defined by the DoE approach (figure 3).

The third expertise involved to achieve robustness of the single-use bag products is related **to the welding, sealing and bag making** competence. The bag making experts can optimize the welding, sealing

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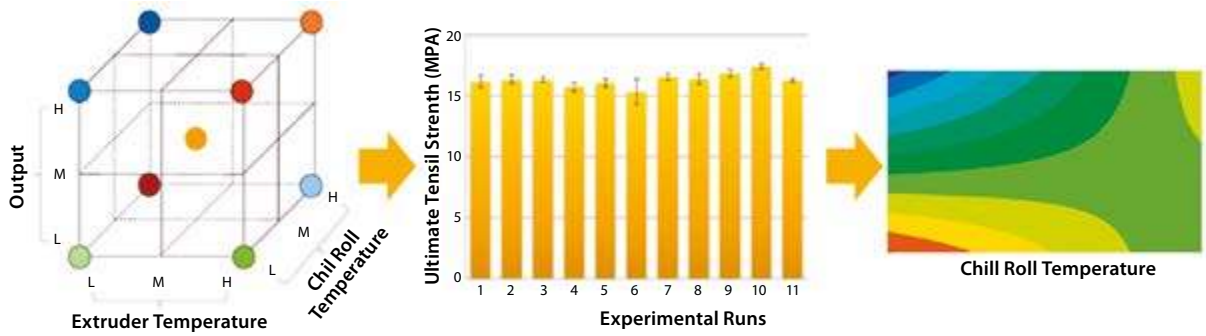

Figure 3: Example of a DoE experiment on film extrusion design space using the ultimate tensile strength as a criterion (MODDE Software).

and bag manufacturing process and can qualify its consistency based on a process risk analysis. The knowledge of process experts, scientists and production engineering provides the adequate level of process understanding. Welding and sealing critical process parameters such as temperature and time are included into the QbD approach, the operating ranges need to be tested and routinely controlled to offer process consistency.

Application proven performance

The robustness of PE film and bags has first been demonstrated by means of standard flex durability, tensile strength, elongation and energy at break testing and water burst test. The suitability of single use bioprocessing bags for all types of processing steps of biopharmaceuticals can be further confirmed for some of the most stringent applications like cell culture, liquid shipping, long-term storage or freezing applications. Robustness trials are typically designed to test the product performance under worst case conditions. Some of these tests follow defined guidelines like the ASTM D4169 for shipping applications (5). In this specific case, the test sequence is designed to mimic worst case conditions, e.g. conditions the liquid filled bags might be exposed to during air or truck shipment at several temperatures. This includes handling tests, horizontal impact (figure 4a) or rotational shocks, vibrations (figure 4b), compression or low-pressurized environment. Successful shipping tests demonstrate exceptional robustness of the bags.

Figure 4a: Horizontal impact ASTM D880-92 Method B

Figure 4b: Vehicle vibration ASTM D4728-06 Method A (air spectrum)

Other tests reflect worst case conditions during applications like long-term cell culture trials (6). Extensive qualification tests can be performed on rocking motion and stirred tank single-use bioreactors using worst case parameter settings during extended periods of time. Successful worst case application tests demonstrate the robustness of the single-use cell culture systems (figures 5a & 5b).

In conclusion, lot-to-lot consistency of the robustness of bioprocessing bags together with all other critical quality attributes such as biocompatibility, purity, cleanliness, gas barrier properties and sterility are critical to ensure safe and reliable single-use bioprocessing. Robust and reliable single-use bag performance is achieved by combining material science, film and bag making expertise and in-depth application knowledge with a Quality by Design approach to development of such bags combined with appropriate in-process controls during bag manufacturing. **Robustness** of single-use bags can only be established by the described multidisciplinary approach. It requires a combination of thickness, strength and flexibility of each layer of the PE film. Resin, film and bag expertise together with application knowledge allow to develop an adequate PE structure that offers outstanding robustness of the film, the welds and the bags suitable for all bioprocessing applications in upstream, downstream and final filling.

Qualification test		Proven performance
Standard fex durability of film	→	High resistance to fatigue& pinhole formation during handling, shipping, rocking & mixing
Tensile strength of film & welds	→	High strength of the film & the welds High resistance of bags to breakage under hydrostratic or air pressure
Elongation & energy at break	→	High flexibility, high resistance to fatigue Ease of installation & use
ASTM D4169	→	Film, weld & bag robustness Safe & robustair & truck liquid shipping
In-house water burst test	→	Strengths of film, welds & the overall bag
Extensive worst case testing	→	Robustness for stirred tank & rocking motion bioreactor bags & shipping applications

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Cahier Pratique

Quality by design applied to viral safety of Biologicals: Case studies & workshop discussion summary

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Applying Quality by Design (QbD) to Viral Safety of biologicals has started to be discussed and implemented to evaluate the robustness of the viral clearance of processes while providing a design space where variations can be acceptable. While numerous QbD studies have been initiated to assess the process performances, limited publications are available on QbD application to viral safety. In this article we use examples of virus filtration and chromatography to discuss how knowledge base and industry experience can help design a risk assessment and a QbD approach for virus removal steps in a biological process.

This article summarize case studies discussed in the workshop the A3P congress of Lausanne in April 2015.
This workshop was aiming at introducing the concept of QbD applied at viral safety and discussing real cases and data from LFB Biotechnologies and Merck.

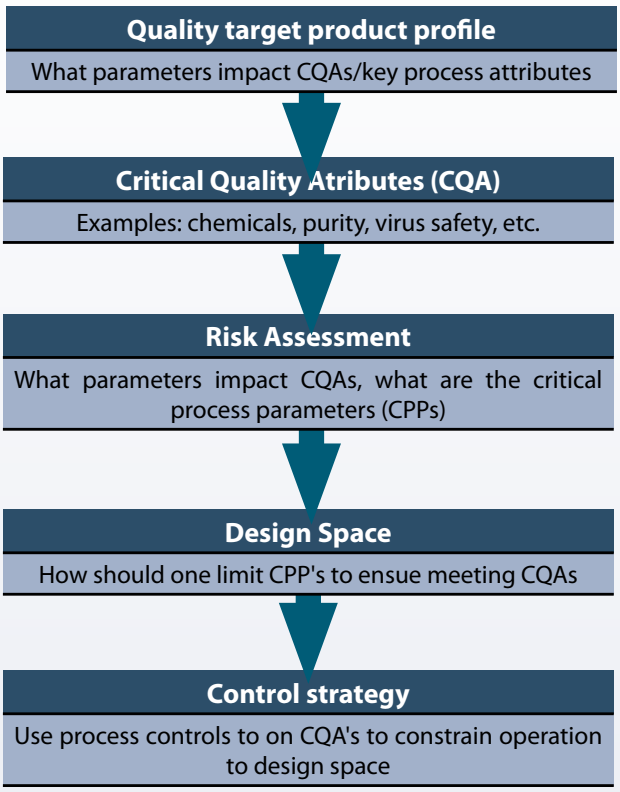

Figure 1: QbD standard sequence

The Quality by Design (QbD) approach has started to be implemented in the biotech industry to ensure the quality of biopharmaceuticals. This initiative allows a thorough understanding of the drug process and the design of a space where variations can occur without altering the quality and the efficacy of the final product. Once validated and approved, the QbD approach may allow more flexibility in manufacturing process changes and reduce change control burden. The concept foundations lay in the International Conference on Harmonization of technical requirement of Pharmaceuticals for Human Use (ICH) guidelines, Q8R2 (Pharmaceutical development), Q9 (Quality Risk Management), Q10 (Pharmaceutical Quality System) and ICH Q11 (development and manufacture of drug substances (chemical entities and biotechnological/biological entities)).

The QbD concept is a stepwise approach and the initial task is to define Critical Quality Attributes (CQA) of the final product, which characterize these attributes and their impact on the product's quality, safety and efficacy (Figure 1). Typically this identification is a result of a thorough risk analysis (according to ICH Q9) and scientific characterization of the product and process. Once the CQAs are identified, the next step is to outline the work flow that needs to be developed in order to ensure the process will manufacture product that meets its product attributes objectives, as defined by the CQAs and eventually the quality target product profile (QTPP). The development of this workflow involves the definition of a design space, a multidimensional area where process parameters can vary and interact in a multivariate fashion. Finally, a control strategy will use process controls on critical process parameters (CPPs) to constrain operations to design space whilst still having acceptable CQAs. The clear advantage of the characterization of this design space is to allow the manufacturer to deal with changes internally and alleviate regulatory approval burden, providing that changes are made within the already validated and approved design space.

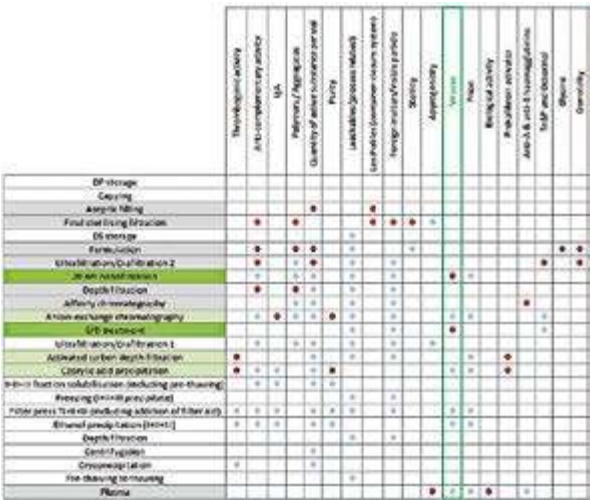

Table 1: Initial Process Risk Analysis: identification of critical steps (example of LFB polyvalent IVIG). Over the 11 critical steps of the process, 2 are specific (dark green) and 2 are contributive (light green) to viral safety.

A process risk assessment is an essential and very effective way to connect the product design, process unit operations and final product performance CQAs. The first step is to evaluate each process step against the defined CQAs in order to identify process steps that would require further characterization. Figure 2 shows an example of the identification process of critical steps for LFB polyvalent IVIG for viral safety of the product, as this is considered an important CQA. The second step is focused upon the potential impact of the process parameters. Establishing a process-design space starts with the definition of CPPs that are likely to have a significant impact on the CQAs and therefore on the quality, safety or efficacy of the product. Any parameter identified having a high potential impact on CQAs is therefore targeted for further study (Table 1). This is accomplished by accumulated knowledge of each step including in-house process development data and from suitable literature. The capacity of a step to inactivate and/or remove viruses remains an important aspect to achieve an acceptable viral safety profile.

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Critical Steps	Contributive studies
Caprylic acid fractionation	Process robustness study
Activated carbon depth filtration	Viral clearance study
S/D treatment	Viral clearance study
Anion-exchange chromatography	Process robustness study **Viral clearance study** Ageing study
Affinity chromatography	Process robustness study Ageing study
Depth filtration	Process robustness study
Virus filtration (nanofiltration)	**Viral clearance study**
Ultrafiltration/Diafiltration 2	Process robustness study
Formulation	Validation batches
Final sterile filtration	Sterile filtration validation study
Aseptic filling	Filling validation study
DP storage	Stability study

Table 2: Process Risk Mitigation Studies (example of LFB polyvalent IVIG)

The assurance and calculation of appropriate viral clearance is an important CQA and should be considered for all products that derived from mammalian cell culture or from biological sources such as monoclonal antibodies, recombinants, vaccines and plasma derived proteins. In the case of products that derive from mammalian cell culture, the rationale of such consideration is first that mammalian cell cultures are known to produce endogenous retroviruses or retrovirus-like particles (RVLP) and second that they have the potential to be contaminated by adventitious viruses during the production or from animal derived raw materials. Blood derived products can carry contamination from the original donors. Regulatory guidelines stress the importance of a multi-layered orthogonal strategy to ensure viral safety of biologicals, which include the

1) The selection of low risk source materials, 2) Appropriate testing and 3) implementation of viral inactivation and removal steps (Figure 2).

Applying QbD in the selection and testing of raw materials[1] and in the viral removal or inactivation steps within the manufacturing process can provide validated assurance of an effective and consistent overall viral clearance strategy.

One can understand that such approach implies a level of investment in the development phase. To date there are limited examples in the literature that describes an approach CQA selection and design space generation dedicated to viral clearance. Genentech (a Roche company) reported the development of viral clearance steps of monoclonal antibodies processes where QbD was applied, specifically on Protein A and anion exchange (AEX) chromatography and on viral filtration [3]. They used design-of-experiments (DoE) and found that simultaneously varying multiple process parameters did not affect the ability of the AEX process to remove endogenous RVLPs or virus models such as xMuLV, MMV (mouse minute virus) and SV40. For Protein A they also did not find that varying process parameters had significant effects thus allowing the establishment of a large design space. Similar findings were reported for viral filtration. Nevertheless, they have observed difference between feedstocks on the AEX and the impact of the loading amount on virus filtration Log Retention Values (LRVs).

Figure 2: Virus Safety Risk Mitigation. Protection of biological processes from contamination by viruses generally relies upon three complementary approaches: sourcing of low-risk raw materials, testing of materials for viral contamination, and treatment of the material to remove or inactivate viruses following the guidelines of the ICH –Q5A [2]

Multiple unit operations will typically contribute to the overall viral clearance, i.e. an amount of log reduction value (LRV) acceptable to ensure the safety of the final product. The workshop "QbD approach applied to viral safety of biological" of the A3 Conference, in Lausanne, in April – focused on the QbD in Viral clearance, specifically chromatography and viral filtration[4]. The objectives of the workshop was to open the discussion on the choice of critical parameters for these unit operations and brainstorm on the approach and methodologies that would support the formation of the design space whilst optimizing resources and time.
Viral clearance studies are costly (typically performed at a contract research laboratory) and time consuming. A full and extensive product and process characterization would be required to define a viral safety design space and this might not be practical with the ressources available. A strategy of establishing a safe space based on the science, risk based selection of parameters (past experience, process step mechanism of viral clearance) and experimental data is rather applicable. Several points that need to be taken into account whilst designing a viral safety study were discussed during the workshop:

Main steps	Contributive steps
Solvent/detergent or detergent treatment	Chromatography (anion-exchange, cation-exchange, affinity)
Virus filtration (nanofiltration)	Fractionation (ethanol, caprylic acid)
Low pH incubation	
Dry heat	
Pasteurization	

Table 3: Viral clearance steps in biological processes

1. Choice of the process step: specific and/or contributive step to virus removal

As mentioned earlier, there are few examples available in the literature of QbD approach applied to viral safety that could allow a clear selection process to be drawn. Validation studies as guided by regulatory documents typically cover a minimum of two different steps to comply with the orthogonal approach requirement. Additional contributions can come from other steps in the process but are not necessary covered by the validation studies, unless the overall LRV achieved is insufficient to guarantee an acceptable safety assurance for marketing authorization. In a biological process, it is considered that viral filtration[5] and viral inactivation (e.g. low pH incubation, Solvent/detergent) - when applicable - are the main viral clearance steps[6] (Table 3). Chromatography steps are also routinely tested for their ability to remove viruses but are generally considered contributive. AEX has been well established as robust and predictable step for removal of viruses including retroviruses and parvoviruses (≥ 4 LRV)[7]. In monoclonal antibodies processes, Protein A has been shown to provide virus reduction capabilities but is generally considered less effective than AEX[8]. Cation exchange chromatography (CEX) is used as a polishing step in some biological processes. The ability of CEX to contribute to virus clearance has been studied by various groups and findings were contradictory. Regardless of the study, it is accepted that a high number of variables directly and indirectly influence viral clearance by CEX including operating pH, elution salt concentration, ionic strength of the equilibration and loading buffer, impurity level, etc[9]. Altogether this knowledge base provides a focus for the QbD approach on the steps that are considered the most effective and typically subjected to virus validation studies for clinical material. For the purpose of the workshop, virus filtration (one main step) and AEX (one contributive step) were selected for further discussion.

2. Choice of Critical Process Parameters (CPPs) for anion exchange chromatography & viral filtration

For AEX, previous studies have demonstrated that feedstock parameters such as pH and conductivity can have an impact on LRV values and can interact with one another in a multivariate fashion[10]. Impurity levels (HCP, DNA) can have a strong impact on virus removal especially at higher distribution coefficient and higher load challenge[11]. It is critical to control the feedstream impurity levels to avoid impurity breakthrough and competitive binding in one hand and assess the impact of residual impurities on virus removal via chromatography. Virus removal of the AEX is generally considered less sensitive to contact time (bed height), flow-rate, resin life time and pooling methods[12]. Finally intrinsic product and virus characteristics such as pI need to be taken into consideration when designing DoE experiments.

For viral filtration, the change in pressure or the interaction of pH and pressure has been found in certain cases to affect the LRV value[13]. This observation has been restricted to only some of the commercially available parvovirus retentive filters, highlighting that a given parameter or the interaction of multiple parameters do not equally impact the virus filters available in the market. Therefore, including these variables in early studies is recommended, especially when filters from different manufacturers are implemented for one

Load (g/L)	Mass throughput	LRV at 50% target volumetric throughput	Final % flow decay	Final pool LRV
2	2.0	N/A	93	≥5.8
7	8.4	≥6.1	86	5.8
15	14.6	≥5.8	87	≥5.8
15	16.3	5.3	89	4.8
25	20.2	5.4	88	5.7
25	20.4	≥6.0	90	≥6.0

Table 4: Assessment of a mAb feed concentration variation on MVM retention of a commercial filter, Viresolve® Pro[17]

Feeds were supplied at 2, 7, 15 and 25 g/L solutions in a phosphate buffer. Before testing feeds were 0.22 µm filtered then prefiltered prior to addition of virus. Devices were challenged at 2 bar with protein solutions spiked with MMV to a target titer of 2 x 106 TCID50/mL. Samples were collected from the filtrate pools at designated volumetric throughput and samples were assayed for infectivity using the standard cell based TCID50 assays. Protein levels were measured in all samples by OD280 readings. All devices were stopped after reaching 80–90% flow decay (approximately four hours after initiation of the test). Effective clearance of MMV was observed across all concentrations with LRVs greater than 4.8, indicating that retention by Viresolve® Pro Devices is not impacted by protein concentration.

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unit operation to enhance security of supply. In a recent study, the commercial parvovirus retentive[14] filter Viresolve® Pro was further evaluated for its parvovirus retention after process interruption where forward pressure was released[15]. Parameters such as timing of the pause, duration of the pause, pore size distribution of the filter membrane and presence of the product were assessed. No significant LRV changes were observed, confirming the initial observations in the previous studies aforementioned.
Bolton et al[16] have observed a relationship between fouling of normal flow virus filter and LRV decline. Using experimental and mathematical models, they reported that the mechanism of LRV decline was due to selective plugging of small pores. As protein solutions may vary in their behavior during filtration the evaluation of the LRV vs flow decay relationship was recommended. Following this observation, studies were performed to assess feed load and mass throughput on commercial filters. An example is provided in the table 4.

The impact of conductivity on virus filtration has also been assessed by different groups. While in some situations it was found that LRV was not impacted by the variation of ionic strength[18] , it was observed that certain ionic strengths were associated to LRV decrease [19].
Filter Shelf life & lot to lot variability are generally assessed to demonstrate the technology robustness and ensure process consistency. Viresolve® Pro retention was assessed across the time using different devices and Phage X174 (Table 5). The study result demonstrated that shelf life was not influencing retention capabilities of the filter. Similar studies were performed to assess lot to lot consistency and results were different across filter suppliers[20].

Device	Shelf life real time (t)				
	0	6 months	1.2 years	2.2 years	3.2 years
0,017 m²	>6.7 LRV	>6.8 LRV	>6.8 LRV	>6.9 LRV	>7.1 LRV
0,07 m²	>7.4 LRV	N/A	N/A	>6.9 LRV	>7.1 LRV
0,22 m²	>7.0 LRV	>7.1 LRV	>7.1 LRV	>7.0 LRV	>7.1 LRV

Table 5: shelf life study of a commercial filter Viresolve® Pro
Viresolve® Pro Modus devices (0.017, 0.07 & 0.22 m2) were spiked with Phage X174 in 50 mM sodium acetate, 100 mM NaCl, pH 5.0, at flow rate of approximatively 10 L/m2 at 30 psi and TCID50 values were recorded for each experiment. N/A indicate absence of data.

Table 6 is a summary of proposed parameters to include in a QbD approach for AEX & virus filtration unit operation development.

Key Design Space Parameters	
AEX chromatography	Virus filtration
Feedstock pH	Feedstock pH
Conductivity (mS/cm)	Conductivity (mS/cm)
Buffer pH	Pressure & process interruption (Bar or Psi)
Impurity level	Load (L/m² or Kg/m²)
Product concentration (load density g/L)	Product concentration (g/L)
Flow rate (CV/hr)	Flow decay (% plugging)
Column volumes (Cm/h, elution, washing, loading)	Filter shelf life
Temperature °C	Filter lot

Table 6: Proposed parameters to include in the risk assessment phase

3. Univariate or multivariate studies ?

During the workshop, the rationale behind conducting univariate or multivariate studies was discussed. A univariate study performed by Genentech was reported and was restricted the protein load[21]. This approach was more considered for business risk mitigation rather than for the viral safety risk. It was proposed that multivariate DoE studies allow to assess the interaction of some parameters and allow the reduction in the number of studies. In a recent collaborative work between Merck and IBET to develop an insect cell culture derived virus-like particle vaccine process, AEX chromatography resins were evaluated for the removal of residual impurities including baculoviruses[22]. DOEs were conducted while varying two parameters: salt concentration and flow rate to assess the VLPs recovery vs Baculovirus removal. The study showed an interaction between the two parameters and allowed to the identification of the best conditions to achieve desired recovery/removal ratio (Figure 3).

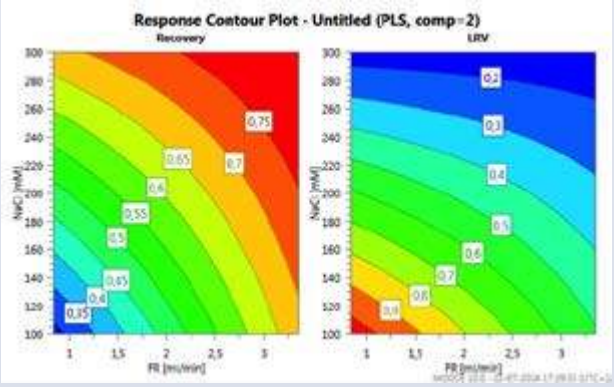

Figure 3: DoE of flow through & Salt conditions for the purification of a Vaccine VLP candidate using Fractogel TMAE® anion exchange chromatography resin. Inputs: load NaCl (100/200/300 mM) and flow rate (100/200/400 cm/hr); Responses: % VLP recovery and Baculovirus LRV

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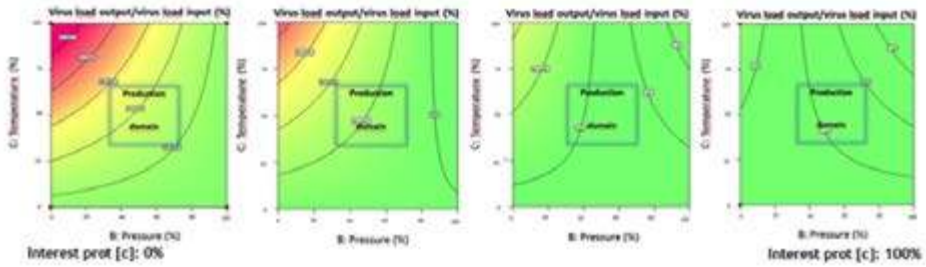
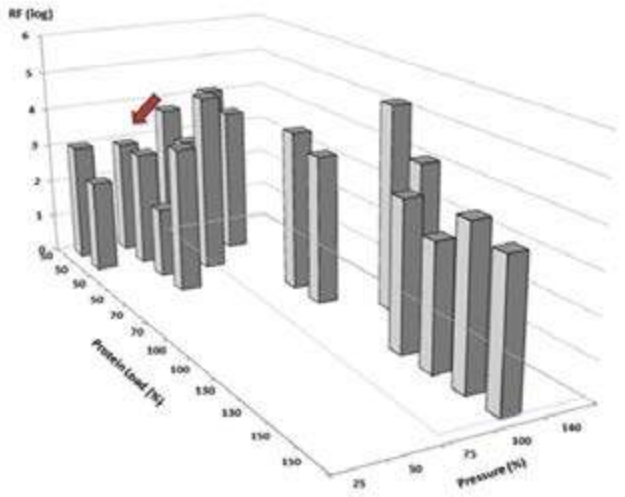


Process parameter	Critical	Target values (%)	Tested domain (%)	PAR values (%)
Volume load	No	50	0 - 100	0 - 100
Interest protein concentration	No	40 - 90	0 - 100	0 - 100
Pressure applied to the filter	No	50	0 - 100	0 - 100
Temperature	No	75	0 - 100	0 - 100

Figure 5: LFB Case study 2 with a plasma derived product – Parvovirus retentive filter and 4 parameters modified in combination

An LFB case study was presented where the effect of two factors (protein load and pressure applied to the filter) was evaluated on filter parvovirus removal (Figure 4). It was postulated that these two parameters could impact virus removal during the parvovirus filtration step as mentioned in the previous paragraph. These potential critical process parameters (CPPs) were therefore retained for following studies. The protein load was demonstrated not to impact on virus removal. In contrast, a trend toward lower log reduction factor was observed at lower pressures (arrow in the figure 4), suggesting an impact of lower pressures on filter parvovirus removal. Process limits have been, therefore, adjusted accordingly.



Process parameter	Critical	Target values (%)	Tested domain (%)	PAR values (%)
Protein load	No	70	0 - 100	0 - 100
Pressure applied to the filter	Yes	90 - 110	0 - 100	170 - 140

Figure 4: LFB Case study 1 with a plasma derived product – parvovirus retentive filter and 2 parameters modified in combination

Accomplishing multivariate studies requires a significantly greater upfront investment in development than does the traditional approach. However, the benefits include operational flexibility, as future modifications to the purification method that remain within the process design space are considered non-notifiable changes and do not require additional viral clearance studies. In addition, the impact of excursions during a production run can be confidently evaluated, reducing clinical and financial risk.

4. Virus models

The choice of virus models for virus removal validation studies derive from regulatory guidelines and common science and relates to the nature of the process. For instance, monoclonal antibodies are typically produced from CHO cell cultures, hence the choice of models such as MMV and xMuLV for the validation of virus removal. For processes that derive from human plasma, blood or urine, or cell culture derived vaccines, other relevant models are used. While the rationale of such choice is clear and understood for virus validation studies, their usage becomes a limitation factor for large DoE and QbD studies. Indeed, not all manufacturers have dedicated laboratories or personnel and outsourcing such studies can be costly.

In their study on AEX chromatography, Genentech proposed to use RVLPs for extensive DoEs. The preparation and the manipulation of RVLPs is less cumbersome and constrained than virus models like MMV and xMuLV. For the same reason, most filter manufacturers use phage models to characterize their virus filters. These models, allow more extensive studies and are relatively economic. The outcome of the workshop was that RVLPs or phage could be used for a first set of DoE studies. Subsequently depending on the result of these studies, a second set of smaller amount of experiments could be run with models that are accepted by the regulatory authorities.

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5. Quality status of the studies: Should the studies follow Good Laboratory Practice (GLP) or Good Manufacturing Practice (GMP) or others?

Viral clearance studies are most of the time performed by a Contract Research laboratory and follow the principles of Good Laboratory Practice (GLP). Viral clearance robustness or QbD studies applied to viral safety are typically carried out at later stage of the product lifecycle, especially for marketing authorization. At this stage, a fully GMP-compliant validated production process is in place, viral safety studies performed at this stage should be therefore GMP-compliant. However, the cost of viral clearance studies being high, their feasibility within a QbD approach might be limited for some companies. Other strategies can be put in place to overcome this situation. During the workshop, it was discussed that one option could be to run the scaled-down viral safety QbD studies (spiking and sample generation) under an internal quality system ("GMP-like") and samples can be titrated either under internal quality system approved assay (Polymerase Chain Reaction (PCR)) or at a contract lab (infectivity (TCID50)). Another option could be the generation of data (screening) with a nonhazardous, non-mammalian virus models (i.e. bacteriophages). The use of bacteriophages allow the generation of a large amount of data with relative speed and cost-effectiveness and this can be performed under GLP or non-GLP conditions either in internal lab or at the supplier of the support (filter, resin, etc.). Ultimately, the approach should be recommended and accepted by the regulatory approval entities taking into considerations the points aforementioned.

6. Statistical methodologies and tools for interpreting large amount of data

The final step of a QbD study is the interpretation of the large amount of data, and this process is not trivial. Indeed, appropriate statistical support or software should be available to provide a structured and organized method to determine the relationship between factors (CPPs) and the outcome (LRVs). This appropriate statistical support should also provide confidence on the interpretation. The question during the workshop was if there is available software which provides confidence on the interpretation of LRVs.

A second LFB case study assessed the effect of four factors (load volume, protein of interest concentration, pressure applied to the filter and temperature) on parvovirus removal (Figure 5). This case highlighted the practical difficulties in interpreting the data, especially due to the use of two statistical methods (Poisson distribution when no virus is detected and Spearman Kärber method when virus is detected). Indeed, two factors seem to have a very minimal impact on virus removal (lower protein feed concentration and lower pressure applied to the filter). This minimal impact is mainly due to detection of virus particles; however, the log reduction factors obtained in these conditions are not statistically significant and very close to the value of the standard condition. It was therefore concluded that the process parameters are non-critical on the tested domain.

7. Group discussion outcome: QbD application to virus filtration study design

Final workshop discussions focused on viral filtration process step. Table 7 provided hereafter is a summarized proposal to help design and support QbD for virus removal filters.

Pathogen agent	A first screening of the parameters with a phage, then selected critical parameters with a parvovirus
Selected parameter	Protein load, load volume, filtration time, pressure, process interruption selected as the main critical parameters. Temperature, pH, concentration, filter lot (data from the supplier) considered less critical
Univariate or multivariate	Multivariate (max. 3 parameters in combination)
Number of runs	Duplicate run for center point and single run for the other points.
Virus titration assay	Infectivity ($TCID_{50}$)
Quality status of the assay	Non-GLP with phage in a first stage and GLP with parvovirus with identified CCPs
Statistical method	Convenient software needed

Table 7: Summary of the group discussion related to parvovirus retentive filter design studies

Conclusion and perspectives

The industry is embracing the QbD approach and its application to virus safety is emerging. Upfront work and solid filtration knowledge is needed to understand how variations in-processing parameters impact the capacity of the manufacturing process to remove or inactivate a potential contamination and thus to define a proven design space. Knowledge base and validated work can allow narrowing down the exploration area. The role of the filter or chromatography resin (or any other technology) supplier is considered key as he can provide robustness studies that help this exercise. Through this approach the risk can be managed effectively and viral safety can be achieved. Still, the workshop outcome highlighted the difficulty to initiate large DoEs and the selection of relevant parameters and combinations as most of the recent works were based on historical experiences and knowledge bases. Moreover, using economical models might be a solution to alleviate the cost of such investigational studies. Finally, because such QbD approach has to be approved by regulatory bodies, more guidance will be needed in the respect of the application of QbD to viral safety to follow common procedures and standards.

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Cahier Pratique

Mass spectrometry as a powerful tool for the characterisation of monoclonal antibodies in the context of comparability studies.

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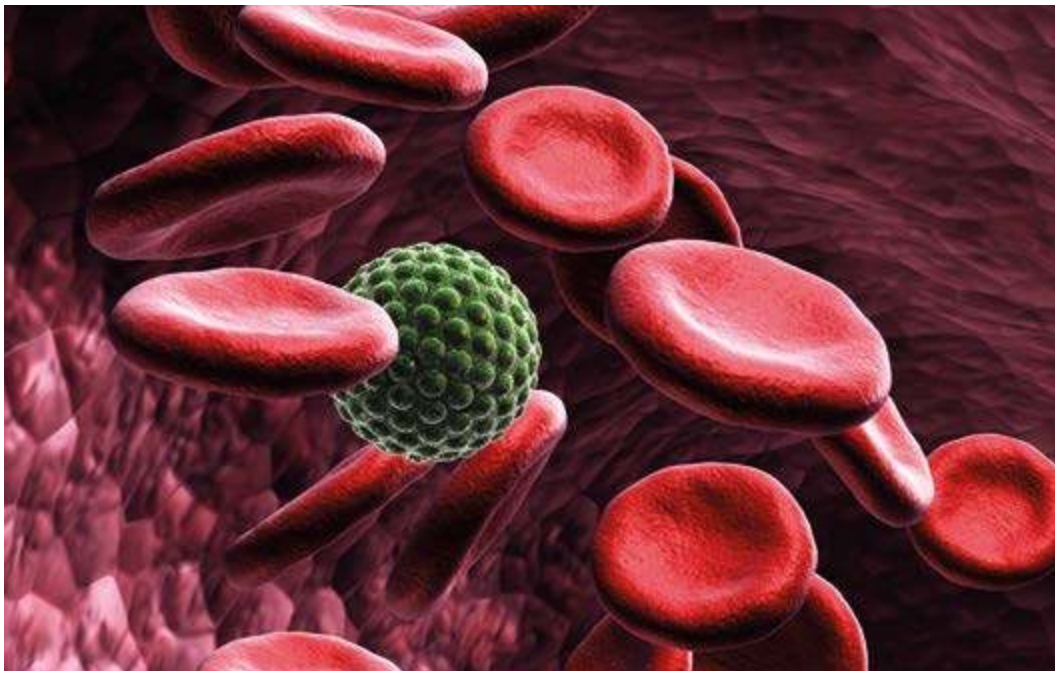

Due to their mode of production, monoclonal antibodies are complex and heterogeneous molecules.

When changes are made to the manufacturing process of a therapeutic monoclonal antibody, an extensive comparability study has to be performed to comply with the requirements of ICH Q5E guidance[1]. These studies usually require the use of many orthogonal analytical techniques in order to fully characterise the different variants.
In this article, the use of mass spectrometry as a powerful tool for comparability studies is presented, with applications of the methods to a commercial product: Humira® (Adalimumab).

Characterisation of intact antibodies

The simplest way of analysing monoclonal antibodies by mass spectrometry is to measure the molecular mass of the intact protein. This test can provide useful information on the identity of the protein, but also on the main glycosylation profile.
The sample is first desalted online by liquid chromatography with a short reversed-phase column to get rid of non-volatile salts. The electrospray mass spectrum obtained is an envelope composed of the different charge states of the protein. It is deconvoluted to yield a spectrum that is more readily interpretable (see Figure 1).

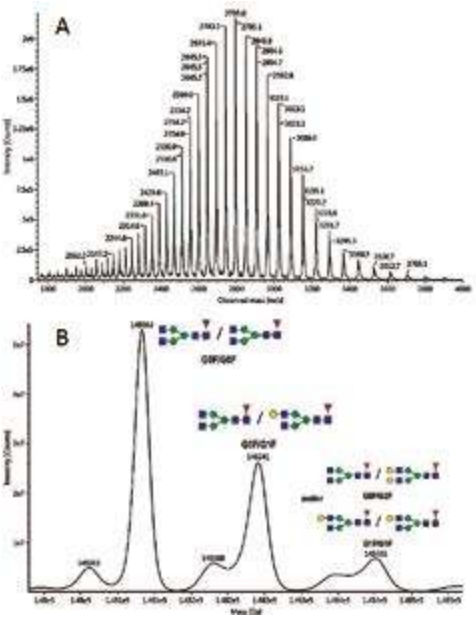

Figure 1: Electrospray mass spectrum (A) and MaxEnt® deconvoluted mass spectrum (B) obtained for intact Adalimumab

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On the deconvoluted spectrum, the glycosylation profile can be easily determined. The main glycoforms observed on Adalimumab are G0F/G0F and G0F/G1F. The mass accuracy is usually below 20 ppm.
In order to simplify the spectrum, deglycosylation can be performed. The corresponding mass spectrum is presented in Figure 2. As the spectrum is simplified, other modifications such as C-terminal lysine clipping can be easily observed.

Analysis of subunits after IdeS digestion

Although intact mass measurement is fast and easy for antibody identification and glycoprofile determination, improved resolution is often valuable, both in terms of chromatographic separation and mass spectrometric determination.
IdeS enzyme is an efficient tool for monoclonal antibody characterisation by mass spectrometry, as the fragments produced have molecular masses of ~ 25 kDa, which allows their analysis by LC/MS with excellent chromatographic resolution and mass accuracy [2].
An example of a chromatogram obtained for Adalimumab after IdeS digestion, with or without further reduction of disulfide bridges, is presented in Figure 3. Molecular masses are measured with a mass accuracy below 1 Da. Thanks to the good chromatographic resolution, variants can also be separated and quantified, such as N-terminal pyroglutamic acid, aglycosylated variants, or oxidised species.

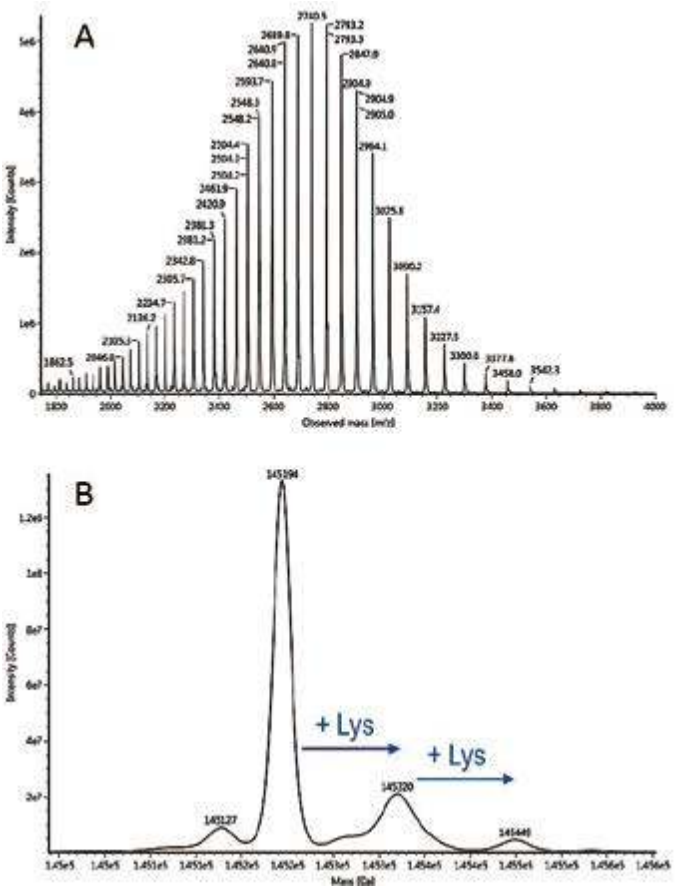

Figure 2: Electrospray mass spectrum (A) and MaxEnt® deconvoluted mass spectrum (B) obtained for N-deglycosylated Adalimumab

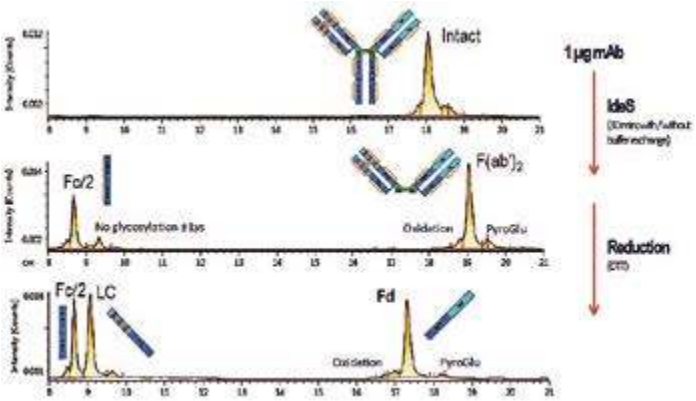

Figure 3: LC/MS analysis of an Adalimumab sample digested with IdeS enzyme then reduced with DTT

Identity testing and targeted purity profiling of antibodies by peptide mapping

The peptide mapping strategy involves the use of a specific protease (e.g. trypsin) to generate small peptides, and the analysis of the resulting peptide mixture by LC with UV and/or MS detections.

Advances in both liquid chromatography, mass spectrometry and software now allow the routine analysis of monoclonal antibodies by a peptide mapping methodology to yield a sequence coverage close to 100 %, together with the fine characterisation of post-translational modifications (deamidation, oxidation, glycosylation, N-terminal pyroGlu, C-terminal lysine truncation, etc.).

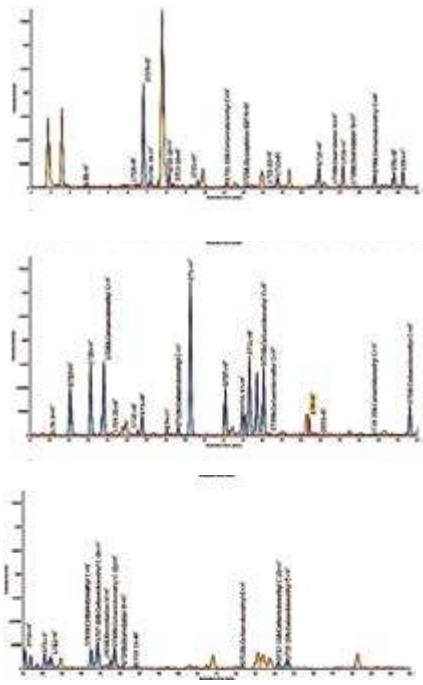

Figure 4: Peptide map of Adalimumab (BPI chromatograms)

The chromatogram obtained for the peptide mapping of Adalimumab is presented in Figure 4. Each peak is identified based on its molecular mass (with a mass tolerance of 5 ppm). The sequence coverage can thus be calculated.

Using this methodology on an Adalimumab sample, we were able to obtain the following results:

- Sequence coverage: 100% (with a mass tolerance of 10 ppm)
- 2.9 % of N-terminal glutamic acid on heavy chain is present as a pyroGlu.
- Heavy chain is mainly present without the C-terminal lysine (89 %)
- Significant deamidation is observed on 152N of the light chain
- The main glycoforms observed are G0F, G1F and G2F, with relative intensities of 75%, 23% and 2%, respectively.

N-glycans profiling by UPLC with fluorescence and high-resolution MS detections

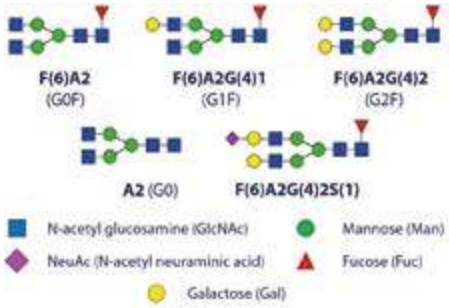

Figure 5: N-glycans commonly found on monoclonal antibodies

Most therapeutic monoclonal antibodies belong to the IgG class and contain a glycosylation site in the Fc region, located on 297N of the heavy chain (see Figure 5).

Glycosylation is a critical quality attribute [3,4], as the N-glycans profile on the Fc moiety influence the binding to Fc receptors, and therefore modulate the ADCC and ADCP activities. Terminal galactose is also important in the complement-dependent cytotoxicity (CDC). Finally, glycans can also have impacts on the safety of the therapeutic antibody.

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UPLC/FLUO of 2-AB labelled N-glycans is a method of choice to characterise the glycosylation of a monoclonal antibody, as well as the batch-to-batch consistency, thanks to its high resolution and reproducibility. With an optimised protocol, the whole procedure including data processing and reporting can be carried out within 24 hours (see Figure 6).

The UPLC/FLUO chromatogram obtained for Adalimumab is given in Figure 7.

The main N-glycans detected (representing 95% of the total N-glycans observed) are presented in Table 1.

Conclusion

Comparability studies performed to comply with the requirements of ICH Q5E after a change of a manufacturing process require the set-up of many orthogonal techniques. Mass spectrometry is a valuable tool as it can rapidly give very accurate information with limited amounts of sample. With only a few analyses, detailed information can be obtained on primary sequence and all post-translational modifications, including glycosylation.

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[2] von Pawel-Rammingen, U., Johansson, B. P. & Björck, L. IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G. EMBO J. 21, 1607–15 (2002).

[3] Reusch, D. & Tejada, M. L. Fc glycans of therapeutic antibodies as critical quality attributes (CQAs). Glycobiology 25, 1–45 (2015).

[4] Beck, A. et al. Trends in glycosylation, glycoanalysis and glycoengineering of therapeutic antibodies and Fc-fusion proteins. Curr. Pharm. Biotechnol. 9, 482–501 (2008)

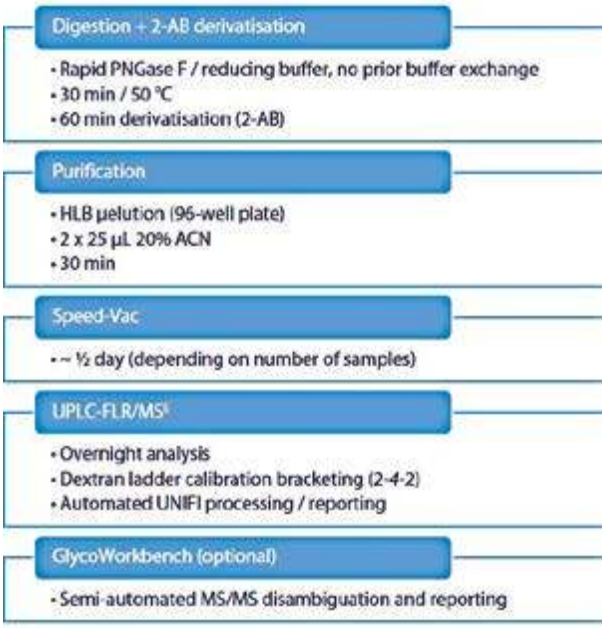

Figure 6: N-glycans profiling workflow

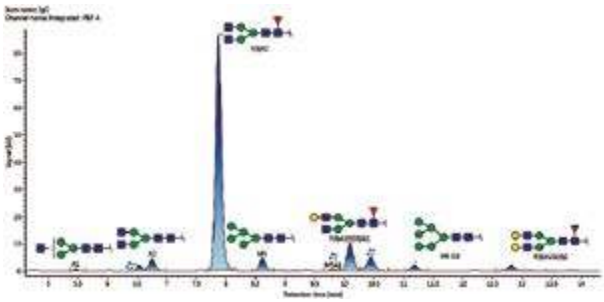

Figure 7: UPLC/FLUO chromatogram obtained for N-glycans profiling of Adalimumab

N-glycan		Mean % amount	% RSD (n=6)
F(6)A2		62.9	1.1
F(6)A2[6]G(4)1		15.2	0.6
F(6)A2[3]G(4)1		6.6	0.3
M5		4.5	0.1
F(6)A2G(4)2		2.0	0.1
M6 D3		2.0	0.1
F(6)A1		1.8	0.2

Table 1: Main N-glycans detected in an Adalimumab sample

Chromatographie Continue :

Solution d'amélioration des performances de procédés et "debottlenecking" des capacités de Bioproduction.

Par Vincent MONCHOIS - Novasep
vincent.monchois@novasep.com

La Bioproduction d'actifs pharmaceutiques nécessite l'emploi de procédés permettant d'assurer leur qualité et leur sécurité ainsi que d'en maîtriser les coûts de fabrication. L'augmentation de la demande et l'apparition des biosimilaires mettent une pression supplémentaire sur ces coûts. L'accroissement de l'échelle de production peut être atteint sans augmentation significative des coûts spécifiques. En effet, l'ingénierie des lignées cellulaires, l'optimisation de la composition des milieux de culture et le développement de nouvelles méthodes de culture en bioréacteur (i.e. culture à haute densité en continu) ont permis d'augmenter la productivité spécifique.
En revanche, la mise à l'échelle des procédés de purification continue à s'accompagner d'une augmentation au moins linéaire des coûts. Le développement de solutions technologiques pour la purification des bio-molécules reste donc un enjeu fondamental pour la croissance du marché biopharmaceutique.

Enjeux liés à la séparation des actifs biopharmaceutiques : Focus sur la chromatographie

Lors de la fabrication d'un actif biopharmaceutique, le procédé de séparation est fondamental car sa mise en œuvre permet d'atteindre les spécifications attendues pour en assurer sa qualité et son activité.

La chromatographie en est une étape clé. Elle permet de séparer la molécule d'intérêt au sein d'un mélange du fait de différences de comportement de ces molécules présentes dans une phase mobile (i.e. une phase liquide dans les procédés de bio-séparation) vis-à-vis d'une phase stationnaire (i.e. une résine packée dans une colonne). Afin de purifier cette molécule d'intérêt, un cycle

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de chromatographie se décompose en plusieurs étapes. Par exemple, dans le cas de la purification des anticorps monoclonaux (mAb), leur capture est réalisée par affinité sur une résine composée de protéine A et un cycle est décomposé en 5 étapes : chargement de l'échantillon, lavage de la colonne pour retirer les espèces non retenues, élution de la molécule d'intérêt, régénération et équilibration. Le dimensionnement du procédé résulte de la capacité ($kg^{molécule}$ / kg^{phase}) et de la sélectivité de phase stationnaire, du nombre d'étapes à opérer au sein d'un cycle ainsi que de sa durée. La limitation principale réside dans le fait que la capacité varie inversement à la vitesse. La mise à l'échelle du procédé est classiquement réalisée en augmentant le volume des résines tout en multipliant le nombre de cycles (mode "batch"). Ainsi, les coûts augmentent au moins proportionnellement en y associant l'augmentation des volumes de tampons et de la taille des équipements (taille des colonnes et des équipements de pompage).

La chromatographie continue : un recul de 50 ans dans l'industrie

Pour répondre à cette problématique économique, des procédés de chromatographie en continu ont été implémentés à l'échelle industrielle pour remplacer la chromatographie en mode "batch". La chromatographie continue est ainsi apparue dans l'industrie pétrolière dans les années 60 sous la forme du procédé de chromatographie à lit mobile simulé (SMB). L'enjeu était alors de produire à l'échelle industrielle, le p-xylène de haute pureté en le séparant de ses isomères. Dans les années 70, l'industrie agro-alimentaire appliqua cette approche à la séparation des molasses de cannes à sucre et de betteraves en développant le SSMB (Sequential Simulated Moving Bed), procédé moins rigide et plus performant que le procédé SMB. Les années 90 marquent l'arrivée de la technologie dans l'Industrie Pharmaceutique pour répondre à l'augmentation du nombre de molécules chirales. Ainsi, des procédés de chromatographie continue haute-performance multi-colonnes en boucles fermées sont employés pour la production de lots commerciaux de molécules chirales telles que le Keppra (UCB) ou le Zoloft (Pfizer).

Application de la chromatographie continue à l'industrie biopharmaceutique : exemple de la Technologie SMCC

Pour répondre aux enjeux économiques liés à la croissance du marché des biomolécules, les mAbs en particulier, l'application de chromatographie continue semble incontournable.

La chromatographie séquentielle multi-colonnes ou SMCC représente, par exemple, une alternative aux procédés traditionnels de capture en "batch", comme dans le cas de la capture d'un mAb sur une résine protéine A. Le SMCC est un procédé de chromatographie continue en boucle ouverte permettant la séparation de différents composés grâce à l'emploi de différentes colonnes (2 à 6 généralement).

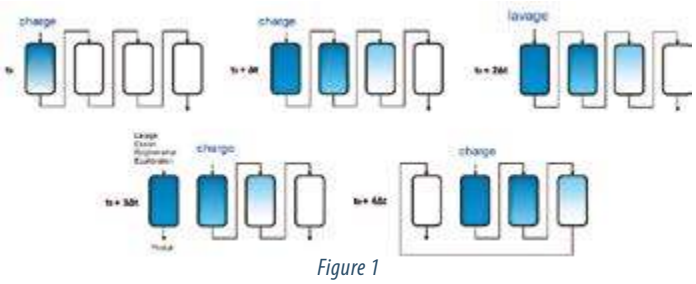

Figure 1

Son principe de fonctionnement est le suivant (Figure 1):

- La charge (par exemple, le milieu de culture contenant l'anticorps) est injectée dans une 1ère colonne, les colonnes étant connectées entre elles. Lorsque la capacité maximale de la 1ère colonne est presque atteinte, la capture se poursuit sur les colonnes suivantes. Une fois la capacité maximale atteinte, une étape de lavage est introduite afin de récupérer tout le produit provenant du volume interstitiel sur la colonne suivante.
- La 1ère colonne est alors déconnectée et le cycle se continue pendant que le chargement se poursuit à partir de la colonne suivante.
- A la fin du cycle, la 1ère colonne est reconnectée et le procédé se répète en partant de la 2ème colonne.
- Ces séquences sont répétées pour atteindre un régime permanent au cours duquel l'injection de la charge est réalisée en continue.

Cette technique permet de maximiser l'utilisation de la capacité de la résine sans perte de produit d'intérêt et d'opérer. En permettant d'accéder à la capacité totale (ou capacité statique non liée à la vitesse), cette technologie permet d'opérer à débits plus élevés qu'en chromatographie "batch".

A l'échelle industrielle, le SMCC permet, à travers l'augmentation de la capacité de réduire les volumes de résines et de tampons et ainsi de limiter la taille des équipements : des gains d'un facteur 2 à 4 peuvent être ainsi observés (Figure 2). Avec l'augmentation des débits rendue possible, la productivité ($kg^{produit}$ / kg^{phase} / 24h) est aussi considérablement améliorée.

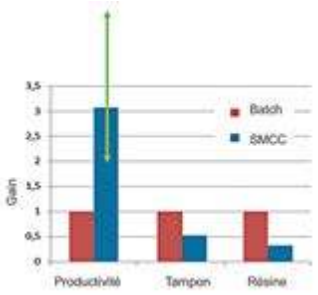

Figure 2

Conclusion

Pour l'industrie biopharmaceutique, l'intégration des techniques de chromatographie continue, telle que SMCC, est essentielle pour que les procédés de séparation atteignent les coûts de revient attendus. Par ailleurs, de l'augmentation de la performance qui en résulte, l'utilisation des capacités en est optimisée, les rendant plus flexibles par rapport à un marché qui évolue.

Protein A Affinity Chromatography for Efficient Fab Purification.

By Melissa HOLSTEIN & Andreas STEIN - MERCK

Fabs, or fragments of antibodies with antigen binding, offer several advantages over monoclonal antibodies (mAbs) including binding to inaccessible epitopes, tissue and tumor penetration, and improved manufacturability. While the purification of these molecules can be achieved through the use of custom affinity resins, separate resin types are often required for different subclasses of Fab molecules. A more widespread approach could be the implementation of generic, commercially available Protein A affinity resins for Fab purification. Protein A resins are often used in antibody purification to take advantage of the strong interaction between staphylococcal Protein A and the Fc region of IgG. Staphylococcal Protein A can also specifically interact with Fab derived from the V_H3 family (Bouvet, 1994; Roben et al., 1995). By leveraging these types of interactions, Fabs may be purified using affinity chromatography resins in a way that is applicable to a wide range of molecules.

In this study, a variety of commercially available affinity resins were evaluated for binding to two Fab molecules. A description of the resins is provided in Table 1. Figure 1 depicts the affinity binding regions on a typical monoclonal antibody, along with kappa and lambda chains. Fab (lambda) binding agarose was used as a negative control since both Fabs used in these studies were based on kappa binding domains.

Resin	Molecule	Base Matrix	Fab Binding
Protein A based agarose resin	Protein A	Cross-linked agarose	VH3
ProSep® Ultra Plus resin	Protein A	Controlled pore glass	VH3
ProSep®-vA Ultra resin	Protein A	Controlled pore glass	VH3
Protein L based agarose resin	Protein L	Cross-linked agarose	VL
Fab (kappa) binding agarose resin	Recombinant protein (Mr 13,000)	Cross-linked agarose	Kappa light chain
Fab (lambda) binding agarose resin	Recombinant protein (Mr 13,000)	Cross-linked agarose	Lambda light chain

Table 1. Description of affinity resins used in Fab binding evaluation.

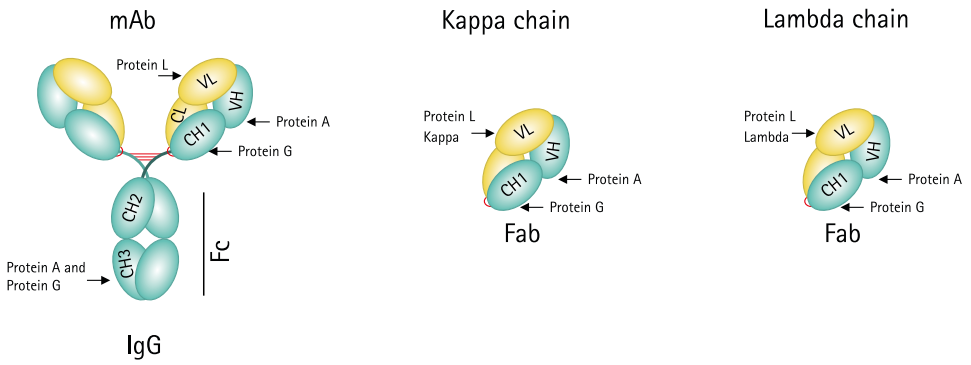

Figure 1. Visual representation depicting affinity binding regions on a typical monoclonal antibody and kappa and lambda chains.

Methods

A purification method was developed for evaluating the dynamic binding capacity (DBC), yield, and purity of the affinity resins described in the introduction. Two Fab feeds were used in these studies. A "Fab01" feed was an Escherichia coli extract and had a titer of 0.7 mg/mL. A "Fab05" feed was purchased from a commercial source (Wacker ESETEC®) and consisted of a Fab molecule expressed in E. coli. The titer of Fab05 was 0.8 mg/mL.

The purification method consisted of standard chromatography steps and common buffer systems described below. Each resin was loaded to 15 mg/mL except for Fab (kappa) binding agarose, which was loaded to 30 mg/mL. Fractions were collected during the loading phase for each resin and analyzed for Fab content in order to determine the DBC values.

Step	Number of column volumes	Residence Time (min)	Buffer
Equilibration	5	3	PBS, 5 mM EDTA, pH 7.2
Load	Load to 15 mg/mL (30 mg/mL for Fab (kappa) binding agarose)	3.7 min for Fab01; 3.0 min for Fab05	Feed; 0.22 µm filtered
Wash	5	3	PBS, 5 mM EDTA, pH 7.2
Elution	8	3	0.1 M citric acid, pH 3.0
Re-Equilibration	5	3	PBS, 5 mM EDTA, pH 7.2

Table 2. Experimental method details.

After determining the DBC of each resin, a second set of experiments was carried out to achieve target loading values and determine the resulting yield and purity. The target loading was 85% of 5% breakthrough. The equilibration, wash, elution, and re-equilibration steps were identical to those described above for the DBC experiments.

The resulting elution pools were collected and analyzed for yield (amount of Fab recovered in elution pool vs. amount of Fab loaded), DNA (PicoGreen® and OliGreen®), HCP (E. coli HCP ELISA), Endotoxin (LAL test), aggregate, monomer, fragment content (size exclusion chromatography HPLC, MS), and SDS-PAGE to confirm breakthrough and purity.

Results

The dynamic binding capacity of each resin was evaluated as described in the Methods section. The results are reported in Figure 2. ProSep® Ultra Plus resin exhibited the highest Fab binding capacity at 30 mg/mL for Fab01. For all resins, the DBC for Fab01 was greater than that of Fab05.
This is likely due to that fact that the Fab05 feed contained a greater amount of impurities which competed for binding sites and reduced the effective binding capacity of the resins. Negligible binding was observed to the Fab (lambda) agarose negative control (data not shown).

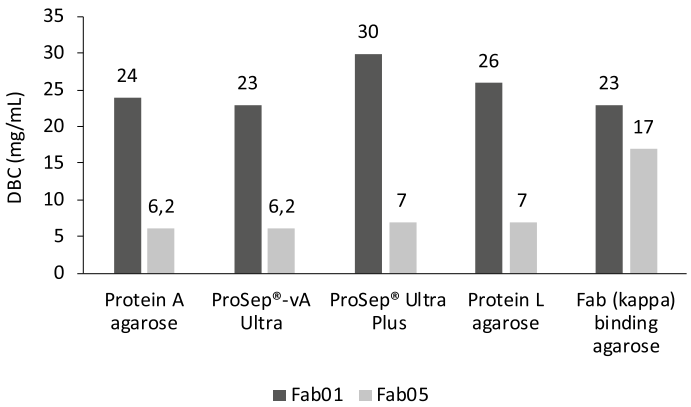

Figure 2. Dynamic binding capacities (DBC10%BT) using Fab01 and Fab05 feeds..

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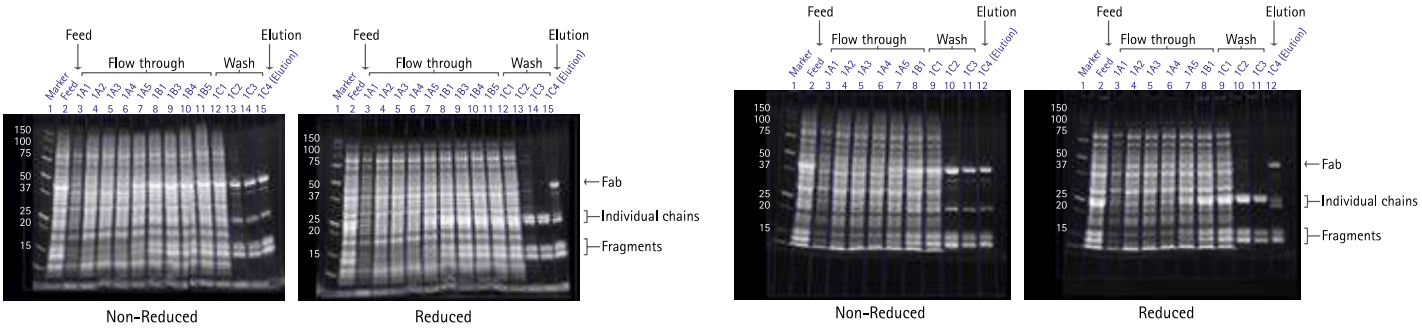

Figure 3. Sample SDS-PAGE gels for ProSep® Ultra Plus (left) and Protein L based agarose resins (right).

SDS-PAGE was used to visualize the Fab breakthrough and to check the purity of the resulting elution pools. Samples of the gels are shown in Figure 3. The band corresponding to the molecular weight of the Fab increased in intensity during the flow-through fractions, corresponding to the breakthrough of the Fab. The elution samples showed significant improvement in product purity as compared to the feed. The elution samples from ProSep® Ultra Plus resin and the Protein L based agarose resin appeared to be comparable based on the SDS-PAGE results.

Resin	Yield (%)	dsDNA (µg/mg)	ssDNA (µg/mg)	DNA LRV	HCP (µg/mg)	HCP LRV	Endotoxin (EU/mg)	Endotoxin LRV
Feed	N/A	322	6486	N/A	5485	N/A	6,222,736	N/A
Protein A agarose	99	0.027	0.44	4.2	15.2	2.6	1690	3.6
ProSep®-vA Ultra	97	0.013	0.12	4.7	14.7	2.6	790	3.9
ProSep® Ultra Plus	94	0.014	0.15	4.6	17.8	2.5	1726	3.6
Protein L agarose	94	0.023	0.33	4.3	12.8	2.6	218	4.5
Fab (kappa) binding agarose	89	0.033	0.44	4.2	12.0	2.7	643	4.0

Table 3. Yield and purity data from Fab01 experiments.

Resin	Yield (%)	dsDNA (µg/mg)	HCP (µg/mg)	HCP LRV	Endotoxin (EU/mg)	Endo-toxin LRV	Aggregate (%)	Fragment (%)
Feed	N/A	30.2	1958	N/A	469,930	N/A	N/A	N/A
Protein A agarose	85	< LOD	0.84	3.4	655	2.9	1.2	1.6
ProSep®-vA Ultra	85	< LOD	0.86	3.4	241	3.3	1.0	1.4
ProSep® Ultra Plus	79	< LOD	0.65	3.5	279	3.2	1.2	1.4
Protein L agarose	73	< LOD	0.54	3.6	400	3.1	4.1	1.8
Fab (kappa) binding agarose	>99	< LOD	0.29	3.8	205	3.4	3.3	1.6

Table 4. Yield and purity data from Fab05 experiments.

The yield and purity data from Fab01 and Fab05 feeds are shown in Tables 3 and 4, respectively. The affinity purification processes were found to be highly efficient. The yields were greater than 70 percent in all cases, with many values greater than 90 percent.
The DNA clearance was excellent for all resins, with values lower than the limit of detection (LOD) for the purified Fab05 material from all of the resins. HCP was reduced by greater than 2.5 LRV in all cases, with some resins achieving greater than 3.5 LRV for Fab05. Endotoxin levels were also reduced significantly for both Fab feeds and all resins.

Summary

Fabs offer some advantages over mAbs including binding to inaccessible epitopes, tissue and tumor penetration, and improved manufacturability. The interaction between Protein A and the V_H3 region of Fabs provides an efficient and widely applicable means of purifying Fab molecules. The purification of two Fab molecules from E. coli feedstreams has been demonstrated using a variety of affinity resins. Like mAb purification using Protein A chromatography, the Fab purification process with Protein A resins was found to be highly efficient with significant HCP (> 3.5 log), DNA (not detectable in final product), and endotoxin clearance (> 3 log).
The use of Protein A resins is particularly desirable because these resins have already been widely adopted in many processes and have robust packing, purification, and storage protocols as well as established Protein A ELISA assay possibilities.

Glossary

DBC: Dynamic Binding Capacity
HCP: Host Cell Protein
IgG: Immunoglobulin G
LRV: Log Reduction Value
mAb: Monoclonal Antibody
LOD: Limit of detection

References :

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Enabling Higher Post Protein A Product Purity Using Novel Chromatographic Clarification Approach.

By William Wessel, Ian Collins & Steven Hager - Catalent Inc
Dmitri Smirnov & Alexei Voloshin - 3M Purification Inc

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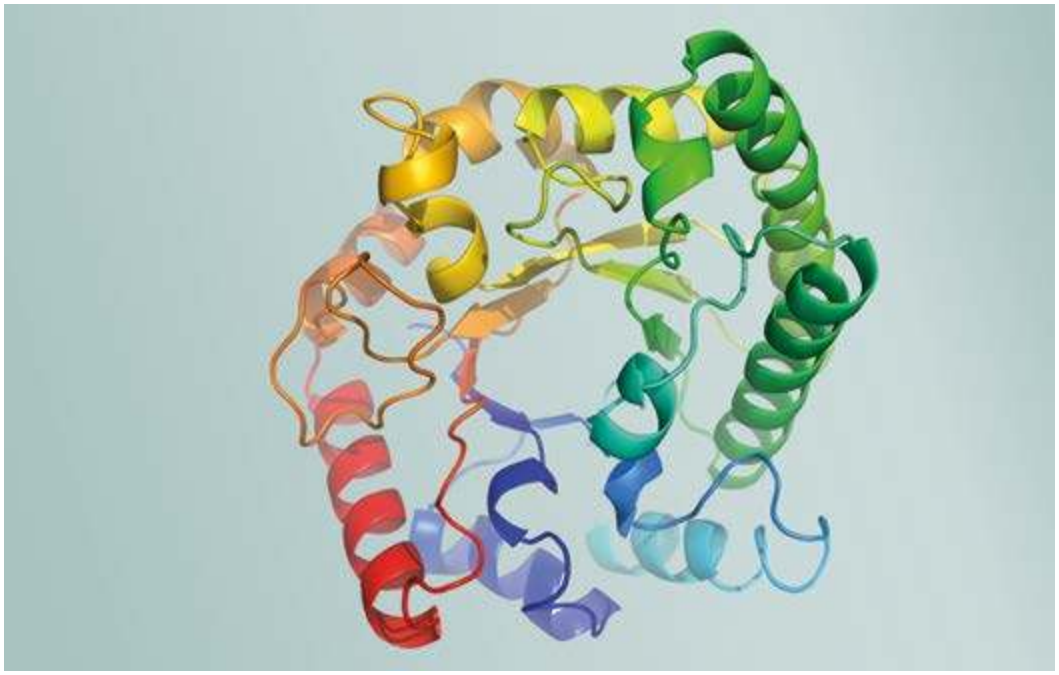

Antibody based therapeutics account for 40% of the entire biotech drug market. In 2012 alone, the total antibody drug production exceeded $50 Billion in sales value[1]. Monoclonal antibodies are becoming the leading driver of the therapeutic product pipelines. Together with the increased number of drug candidates comes the challenge of ensuring that an adequate, low cost, safe, and scalable manufacturing capacity exists and is able to address the ever increasing complexity of these bio-therapeutics.

While a lot of advances and optimizations have been made to the classic antibody production process to make it scalable, reproducible, and commercially viable, the basic structure of the process has changed little and significant challenges remain. Increasing cell densities, longer cell-culture duration, and higher product titers have challenged the basic design of the downstream train to deal with additional cell mass, cell debris, host cell protein, DNA, adventitious and endogenous viruses, and other impurities.[2]

At the front of the purification train is the clarification stage that clears the cells, cell debris, and the other insoluble aggregates. Typically, a combination of the centrifugation, depth filtration, and microfiltration is deployed to reduce the stream turbidity from over 1000 Nephelometric Turbidity Units (NTU) to under 10 NTU [3]. The process stream containing the product is then loaded onto the Protein A column which acts as the primary capture step [4]. Protein A, in turn, takes on the brunt of the work as it is exposed to host cell contaminants, including proteolytic enzymes, a large concentration of impurities that non-specifically bind to the column side by side with the mAb product, and denaturing cleaning conditions after every cycle [5].
As the result, the Protein A capture step is stressed to live up to its promise of purity and robustness, forcing a number of additional steps to be implemented downstream of Protein A.

The recent focus on chromatin and chromatin related impurities [6, 7] only further highlights the importance of developing a strategy for targeting and removal of the bulk of soluble contaminants upstream of the Protein A column.

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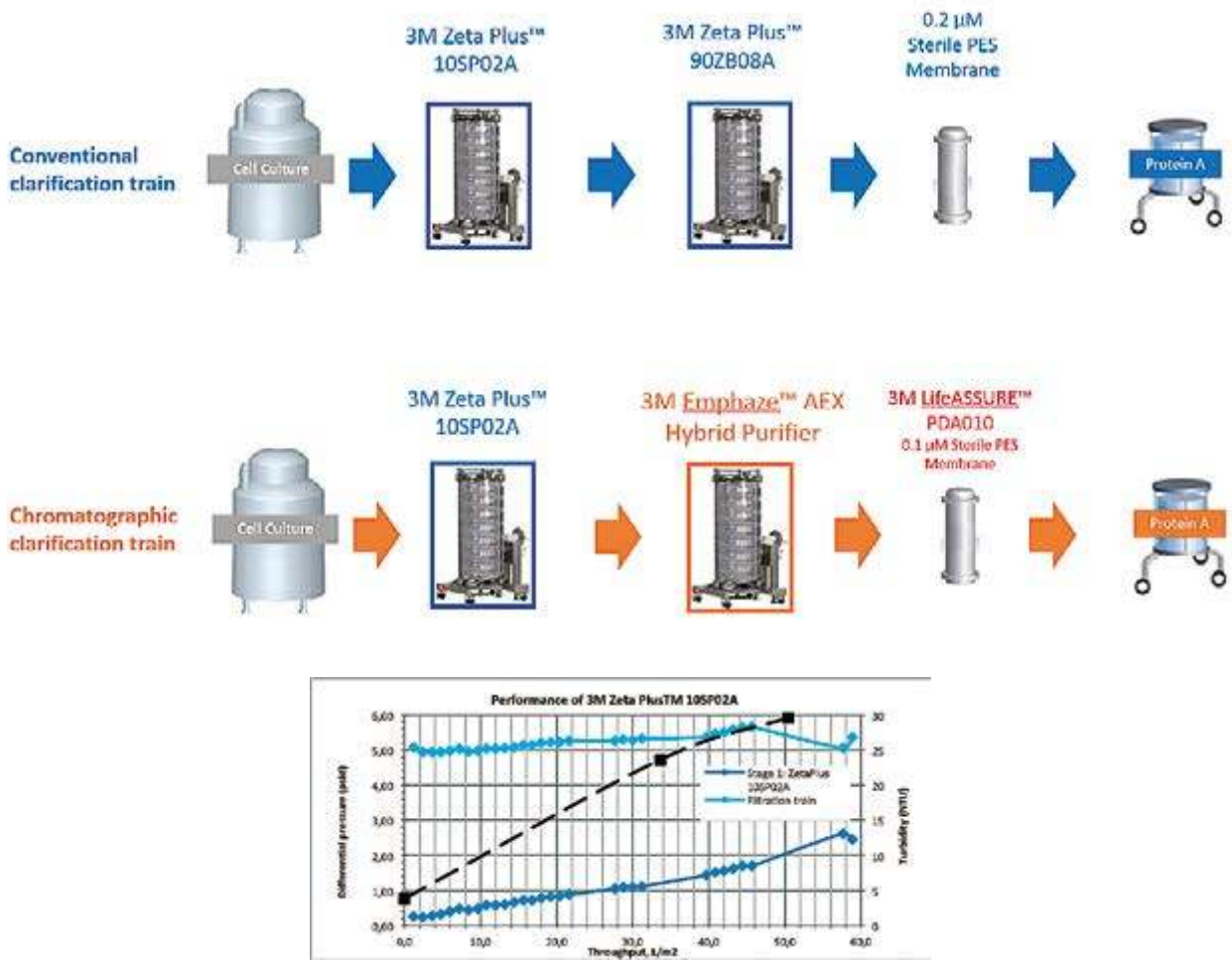

Figure 1

Until recently, efforts to design an effective clarification train that will protect the Protein A column not only from macroscopic debris, but also from high concentrations of soluble DNA and associate HCP contaminants have been complicated by the inability to combine depth filter clarification properties, ion exchange chromatographic adsorption, and bio-burden reduction of a microscopic filter in a robust and cost-effective manner. [8] Traditionally, such implementations would include a depth filter followed by a membrane ion exchange flow-through device, followed by a 0.2 um filter. This, however, is more expensive, adds additional steps, and decreases process robustness.

Recently, Angelines Castro-Forerro *et. al* [9]. demonstrated such a strategy using a 3M Emphaze™ AEX Hybrid Purifier – a functional non-woven based anion-exchange clarifier. This product uses a highly charged Q-functional non-woven polymer matrix (a hydrogel) to clarify the cell culture fluid (CCF) stream as well as to effectively remove DNA ahead of Protein A. The result was > 10 fold reduction of HCP and DNA in the Protein A elution pool. In addition a dramatic decrease in the amount of hard-to-remove HCP and DNA contaminants left on Protein A resin after product elution was observed. If such a strategy can be implemented effectively in the industrial process, it will greatly enhance, not only the affinity capture step efficiency and cycle lifetime, but also has the potential to simplify the polishing train downstream of the capture step [6].

Herein we describe an implementation of such a strategy by incorporating chromatographic clarification through the use of the Emphaze™ AEX Hybrid Purifier in an industrially relevant process. The clarification performance is assessed, as well as its effect on post Protein A HCP and DNA contamination.

Experimental

The standard CHO cell culture (cell density of 2.8*106 cells/ cc, viability of 52 %) expressing a recombinant mAb product (IgG[1], pI = 7.8) was clarified. Depth filtration clarification process, incorporating, either 3M Zeta Plus™ 60SP02A in a single stage, or 3M Zeta Plus™ 10SP02A follows by a 3M Zeta Plus™ 90ZB08A was replaced with a dual stage approach utilizing 3M Zeta Plus™ 10SP02A as the primary cell removal

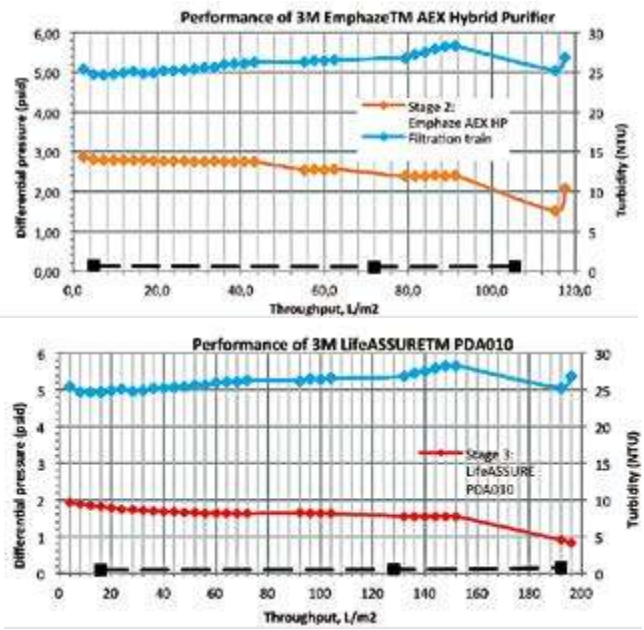

Figure 2

stage followed by the 3M Emphaze™ AEX Hybrid Purifier as the second stage in a 2:1 area ratio respectively (Figure 1).

We also replaced the standard 0.2 µm filter with a 0.1 µm (3M LifeASSURE PDA010) sterile filter. Since the Emphaze™ AEX Hybrid Purifier reduces the DNA down to very low concentrations and contains a 0.2 µm qualifying zone, it offers excellent protection for a 0.1 µm sterilizing membrane filter.

Figure 2 summarizes pressure and turbidity data across the 3 stages of this train (Zeta Plus™, Emphaze™, and LifeASSURE™). The Zeta Plus™ stage behaves in a typical depth filtration manner with linear increase in turbidity and pressure throughout its lifetime. Unlike the Zeta Plus™ however, the AEX Hybrid Purifier offers constant outlet turbidity and pressure.

This is consistent with the fact that the chromatographic clarification relies on capture by charge of the hydrogel rather than by the tortuous path traversal. Thus one would expect that the pressure would remain constant until the charge capacity of the hydrogel is exhausted. This type of approach provides excellent control of and stability during the clarification process.

The pressure across the 0.1 µm stage is also low and constant indicating that AEX Hybrid Purifier provides excellent protection of the sterilizing membrane filter stage. It was previously reported (9) that the AEX Hybrid Purifier removes DNA-protein complexes having hydrodynamic sizes in the range of 100-200 nm and the size of the particles coming out the chromatographic clarification stage was limited to ~ 30-40 nm in that study (IgG1 hydrodynamic radius is 11-12 nm).

Together with the pressure data obtained in our experiments, this would suggest that even a 0.1 µm filter will have, virtually, unlimited throughput and can be sized based on flowrate alone. **This is, clearly, an advantage at manufacturing scale as the typical sterilizing filter stage post clarification is designed based on throughput.**

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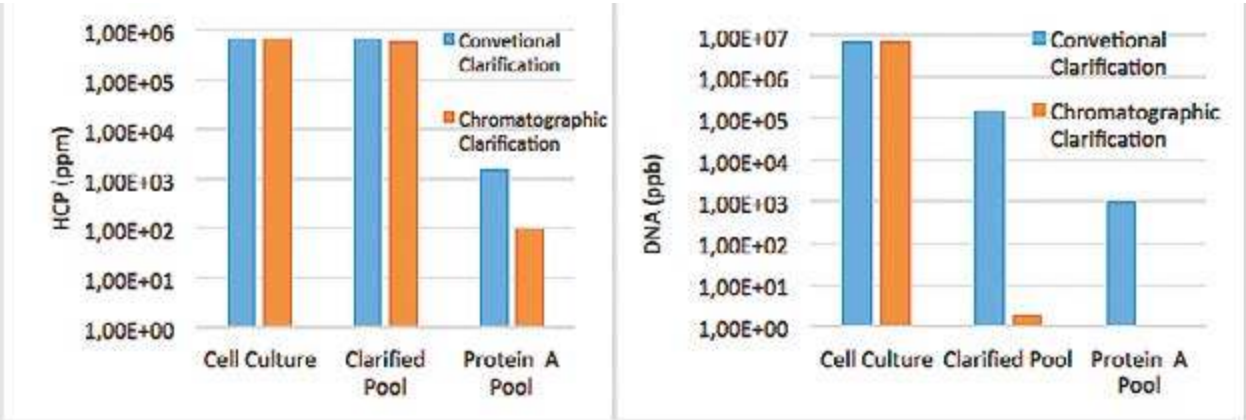

Figure 3

The Clarified Cell Culture Fluid (CCCF) obtained using the chromatographic clarification train was purified on Protein A column (MabSelect, GE) using a standard purification protocol. Figure 3 summarizes HCP (performed via Cygnus CHO HCP kit) and DNA (performed via Q-PCR) at the pre-clarification stage, post clarification stage, and in the Protein A elution pool. What is clearly evident from the data is the high degree of the DNA removal by the Emphaze ™ AEX Hybrid Purifier and very high mAb product purity post Protein A purification. The DNA is cleared to 2 ppb (> 6 LRV), that is at the limit of detection for the Q-PCR. High degree of removal of the interfering DNA contaminant enables qualitatively higher purity in the Protein A elution pool at 1 ppb DNA and 100 ppm HCP. This is the case even when the HCP reduction over the entire clarification train is only ~ 20 %. This is > 10 X improvement in post capture purity compared to the conventional approach and is a step change in the performance of the Protein A step and, thus, the purification process itself.

We then purified 3 additional mAb products using the same chromatographic clarification strategy to understand the consistency of this approach. Figure 4 summarizes the post Protein A purity data. All 3 products were eluted off the Protein A with very high purity at, or near, the quantifiable limit in terms of DNA and HCP content.

Discussion

Dramatic and consistent increase in product purity post capture to the near injectable level brings into view a number of process development paradigms that are critical to the development of the mAb purification processes of tomorrow.

The first is platformability of the mAB purification process. The main premise and driver of rapid bio-pharmaceutical drug development over the last 10 years has been the principle of platforming of the key steps in the discovery-to-clinic-to-manufacturing process.

Antibody scaffold only further facilitated this through a unified bio-pharmaceutical product architecture. Yet, purification process remains, largely, a custom-designed approach for each mAb product, primarily due to inconsistent and relatively low post Protein A purity, which dictates the engineering of the polishing train.

Chromatographic clarification approach solves this problem getting one step closer to building a unified polishing train for every product. Whether this unified polishing train strategy is one process configuration or a rapidly selected set of several standard strategies is immaterial. The high and consistent purity of the product post capture enables a consistent and predictable approach to polishing, and thus, to the entire purification process. This, in-turn shrinks the process development time and accelerates the discovery-to-clinic-to-production pipeline.

The second critical element enabled by the high post-capture purity is a step-change in cost and productivity of polishing trains. The size, cost, and architecture of the polishing part of the mAb purification process is dictated by the amount and consistency of the impurities that are left in the process stream post Protein A. High product purity enables more compact and cheaper polishing trains. This element becomes more critical as the process scale rises and its complexity and cost grow non-linearly.

Since the chromatographic clarification enables more than an order of magnitude reduction in DNA and HCP product stream contaminants, one

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can reduce the size of the required process steps, such as the AEX polishing step, accordingly. In cases, delete comma where viral clearance requirements are abbreviated, such as for preclinical uses, the polishing train may be completely eliminated. At large scales this leads to a significant risk reduction and cost savings. More importantly it enables one to deploy more purification trains into existing facilities, delaying the often politically and financially challenging capital expenditures for construction of new manufacturing plants.

Conclusion

Efficiency of the product capture step has always driven the performance of the entire process. Increases in efficiency of the capture step in the mAb purification is a subject that has been relentlessly studied for the last 10+ years. Genomic DNA and associated chromatin complex has been identified as the likely major component interfering with the Protein A chromatography. While methods, such as cell culture acidification and treatment, do exist to reduce the DNA in the clarified cell culture media, they have, either been limited to academic space, or have shown to be of limited use in the industrial space due to product stability and process control issues.

Here we demonstrate a novel alternative method of chromatographic clarification approach using an anion exchange clarifier 3M Emphaze™ AEX Hybrid Purifier to reduce the DNA down to near the limit of detection at clarification. The result of this DNA reduction produces a step change in the purity of the product eluted off the Protein A column. Unlike other strategies, this approach enables a high degree of DNA reduction in a scalable, flow-through, single use system that is industrially viable and rapidly deployable in a commercial process.

The consistently high purity of the product post capture streamlines development, lowers manufacturing costs and improves process efficiency that comes with high process platformability and robustness.

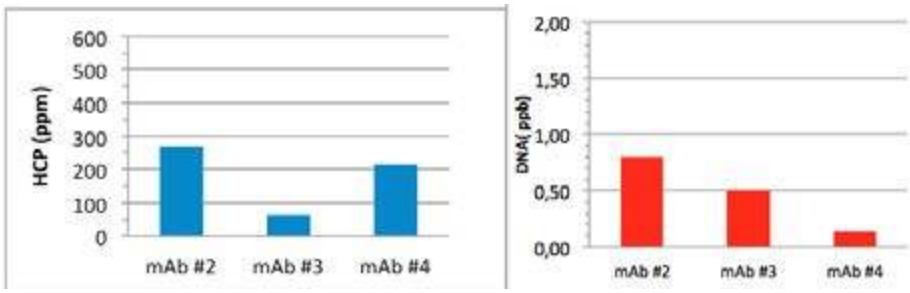

Figure 4

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L'agenda

Événements A3P 2016

Détection des Mycoplasmes
Contrôle des Impuretés
16 JUIN
Villeneuve-la-Garenne, FRANCE
1 Jour, 1 Labo : conférences, visite du site SGS

Forums A3P Belgique
21 AVRIL & 16 OCTOBRE
BELGIQUE
Conférences, visite de site, exposition

Particules Visibles
5 JUILLET
Le Vaudreuil, FRANCE
Conférences, tables rondes, exposition

Forums A3P Suisse
12 MAI & 27 SEPTEMBRE
SUISSE
Conférences, visite de site, exposition

Endotoxines
20 SEPTEMBRE
Lyon, FRANCE
Conférences, tables rondes, exposition

Bioproduction
24 & 25 MAI
Bruxelles, BELGIQUE
Conférences, ateliers, visite de site, exposition

Congrès international A3P
15, 16 & 17 NOVEMBRE
Biarritz, FRANCE
Conférences, ateliers, exposition

Congrès A3P Algérie
10 & 11 MAI
Alger, ALGERIE
Conférences, ateliers, exposition

Forum A3P Maroc
DÉCEMBRE
Casablanca, MAROC
Conférences, ateliers, exposition

Forum A3P Algérie
DÉCEMBRE
Constantine, ALGERIE
Conférences, exposition

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Près de 30 sujets abordés,
répartis en 5 domaines
spécifiques au "Propre et Stérile"
animés par 15 experts reconnus
dans chacun de leur domaine.

Tout le programme sur www.a3p.org

Maîtrise de la Contamination

- Determining Particulate Matter in Liquids: Parenteral & Ophthalmic Products
  Date : 19 avril // MC03
- Essentials of USP Microbiology
  Date : 27 avril // MC04
- Essentials of Testing and Control of Microbial Quality of Nonsterile Drug Substances and Products
  Date : 26 avril // MC05
- La nouvelle version de la norme ISO 14644-1 : Quels changements ?
  Date : 28 avril // MC06
- Elaboration d'un programme de bio-nettoyage en salles propres en environnement BPF
  Date : 23 juin // MC07
- Maîtrise de la contamination en ZAC (stérile et non stérile)
  Date : 28 & 29 juin // MC08
- Analyse du risque sur les étapes critiques de procédés stériles, aseptiques ou à contamination contrôlée : analyse, spécifications, moyens de maîtrise de la biocontamination
  Date : 7 & 8 juin // MC09
- Analyse du risque particulaire dans les produits stériles et injectables : analyse, points critiques et maîtrise, gestion de la criticité des déviations / OOS associés
  Date : 13 & 14 avril // MC10

B.P.F.

- Mise à jour réglementaire et état de l'art pour la fabrication des produits pharmaceutiques stériles
  Date : 31 mai // BPF04
- L'Annexe 1 des GMP Eu : les points critiques, leur analyse et leur interprétation
  Date : 21 & 22 juin // BPF05

Systèmes Informatisés

- L'audit et l'inspection des SI : outils et méthodes
  Date : 20 avril // SIO2
- Validation des systèmes informatisés efficace et efficiente : outils et méthodes
  Date : 19 mai // SIO3
- Évaluation des fournisseurs IT/IS : Outils et pratiques
  Date : 16 juin // SIO4

Process

- Lyophilisation 3 : expertise et maîtrise des procédés et de la qualité
  Date : 14 & 15 juin // PROC03
- Maîtriser les principes de la culture LEAN pour vous et par vous
  Date : 11 & 12 mai // PROC04
- Stérilisation par la chaleur : principes, validation et production Fondamentaux et aspects pratiques
  Date : 24 & 25 mai // PROC05
- Spécificités du marché pharmaceutique japonais
  Date : 1 juin // PROC06
- Travail en campagne en isolateurs de production
  Date : 2 juin // PROC07
- Le Lean : poison ou potion ? Le management du bon sens au service de la qualité
  Date : 8 & 9 juin // PROC08

Qualif

- Bioassay Design, Development, and Validation
  Date : 26 mai // QUAL02

Informations & inscriptions sur www.a3p.org