La Vague

LE MAGAZINE DE LA PHARMA ET DES BIOTECHS

N° 53 | Avril 2017
Trimestriel

Bioproduction

Congrès A3P Bioproduction 2017, les 31 mai & 1 Juin à Lyon

- **Frontière Part I/ Part II des BPF : Modalités d'application aux produits biologiques.**
- **Réglementaire : nouvelle rubrique qui recence les récentes évolutions.**
- **Improving Single Use Bioreactor Design and Process Development.**
- **Qualification approach for the validation of real-word shipping in single-use systems.**

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ISSN 1298-047

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N°53 // Avril 2017

La Vague
Revue trimestrielle N° 53 - Avril 2017

• Editeur
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Prix de vente au numéro : 10€

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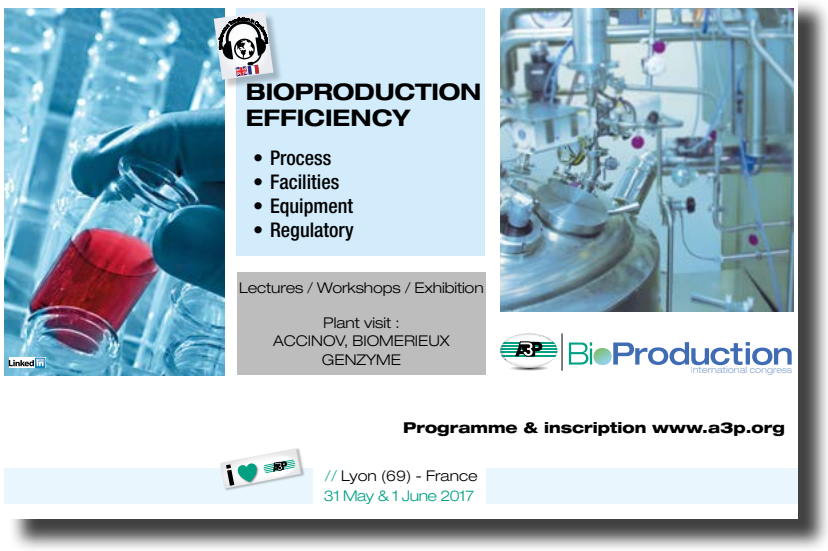
• Impression
2PRINT - 42000 Saint-Étienne

Dépot légal à parution
N° d'ISSN : 1298-047
N° CPPAP : en cours

L'édito

4th Bioproduction A3P Congress



Over the past decade, biomanufacturing has become a strategic driver to scalable and flexible operations, controlled costs.
The advances in manufacturing technologies in particular continuous manufacturing, process analytical technology, single-use systems,
alternative down processing techniques lead to improve the efficiency of the process contributing to cost reduction which becomes a challenge for all the drug makers.

In the upcoming **BIOMANUFACTURING A3P CONGRESS**, on May 31st and June 1st 2017, you will have the opportunity to learn about key trends, methodologies and insights on manufacturing, process development, quality management and compliance of bioproducts.

Who will you meet :
Network with senior VPs, Heads, Directors, Managers and decision makers of leading biopharmaceutical and biotech companies whose responsibilities include Manufacturing, Process Development, Bioprocessing, Cell Culture Manufacturing, Technical Operations, Process Excellence, Quality, Validation, Outsourcing and Regulatory.

Welcome to Lyon on 31 May & 1 June 2017 for its 4th International Bioproduction A3P Congress !

Program & Registration www.a3p.org

Contributeurs

Ils ont participé à ce numéro

Katell MIGNOT
Sartorius stedim biotech

Rédactrice de "Qualification Approach For The Validation Of Real-World Shipping In Single-Use Systems"

Mrs. MIGNOT is Field Marketing Manager for Single Use Fluid Management Technologies at Sartorius Stedim Biotech, based in Aubagne, France. She holds a Master degree in Biochemistry and gained 18 years' experience at suppliers for Biopharmaceutical Industry. She joined Sartorius Stedim Biotech in 2005 and, since 2009 she has been leading a team of engineers who support process design, validation, training and implementation of single-use fluid management technologies in South Europe and MEA areas.
Mrs. MIGNOT is an active member of ISPE, PDA, A3P and BPSA.

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Thermo Fisher Scientific

Rédacteur de "Improving Single Use Bioreactor Design and Process Development – New Research Towards Intensifying Seed-Train and Scale-up Methods Using 5:1 Turn-Down"

Peter LEVISON, PHD
Pall Life Sciences

Rédacteur de "Moving One Unit Operation At a Time Toward Continuous Biomanufacturing"

She holds a PhD in Biochemistry from the UniverDr. Peter Levison joined Pall in July 2003, bringing over 20 years of experience in the biopharmaceutical industry to various positions of increasing responsibility in R&D, technical management and product management.

Sébastien LEFEBVRE
VERDOT Ips²

Rédacteur de "Close Collaboration Maximizes Value of Engineered Solutions and Saves Time in Start-Up"

Sébastien Lefebvre is Product & Application Manager at VERDOT Ips². Graduated in Mechanics and Biology, co-author of 3 patents in Chromatography, with an experience of 25 years in Process engineering.

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Boccard Health & Care

Frédéric CHEVIRON
Boccard Health & Care

Rédacteurs de "Single Use & Stainless Steel: complementarity or clash?."

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 Patrick Hibon de Frohen - Administrateur A3P

Les Biotechnologies : l'avenir pour l'industrie du médicament

Un des axes de développement et de production des produits de santé, sur le plan national, est celui issu des biotechnologies. Or selon une étude de Roland Berger publiée l'année dernière, sur les 130 molécules, issues des biotechnologies autorisées en Europe entre 2012 et 2014, 8 étaient produites en France, contre 32 en Allemagne, 28 au Royaume-Uni et 13 en Italie.

Face à ce constat, le Comité Stratégique Régional de l'Innovation de la région Centre Val de Loire, a lancé dès 2015, une étude portant sur cinq grands domaines potentiels de spécialisation, dont celui sur "les biotechnologies et services appliqués à la santé et à la cosmétique". Celle-ci, confiée à un binôme "industriel/ universitaire", a formulé des préconisations afin de permettre l'émergence d'une stratégie politique et financière afin de rattraper notre retard dans le domaine concerné face à la concurrence internationale.

Ces préconisations reposent essentiellement sur certaines prises de conscience, et donc, à termes, sur des actions concrètes. En effet, les capacités industrielles en matière de santé doivent mettre en œuvre les avancées théoriques. Ceci suppose une articulation efficace entre recherche fondamentale, essentiellement académique, et développement industriel, grâce à des clusters, des pôles de compétitivité ou à des organisations comme l'A3P. D'autres conditions sont nécessaires : trouver et obtenir des fonds capables de financer la recherche et les investissements indispensables pour le passage à la production industrielle (proposition de la création d'un crédit impôt/ investissement), mettre en place des systèmes de veille, de sécurisation et de protection juridique des avancées réalisées, la capacité à faire accepter par les instances nationales (ANSM) des normes techniques et réglementaires nouvelles (c'est l'objet d'un des GIC d'A3P) et enfin, ce qui semble le plus complexe à instiller, une éthique et une culture de l'innovation et donc une propension culturelle à refuser de se reposer sur ses acquis.

Nous sommes, nul ne peut le contester, en situation de "guerre économique". Si celle-ci est une lutte, elle s'appuie sur des armes. **Dans ce contexte, un grand nombre "d'outils" valent comme arme. La première de toutes est sans conteste, la formation ou l'information à travers nos congrès ou journées à thèmes. Dans nos entreprises, en perpétuelle évolution, ces actions contribuent à créer une main d'œuvre et des cadres préparés au changement. L'ensemble des acteurs socio-économiques, à commencer par les industriels eux-mêmes, doivent se donner les moyens des ambitions et des préconisations formulées, au risque que les études évoquées ci-dessus, soient sans lendemain.**

Réglementaire

By AKTEHOM

À ne pas manquer !

Sur la base d'une fréquence trimestrielle, ce point réglementaire a pour objectif de présenter les récentes évolutions réglementaires au regard du cycle de vie du produit. Non exhaustive, cette sélection des parutions récentes se focalise sur les grandes thématiques impactant les métiers pharmaceutiques.

Based on a quarterly frequency, this newsletter informs you about the recent regulatory trends in accordance with the product life cycle. It is a non-exhaustive selection of publications produced during the recent period, focusing on the main topics impacting pharmaceutical activities.

Développement - Development

Publication de textes de consolidation de plusieurs thématiques, notamment en ce qui concerne les produits biosimilaires et l'évaluation des risques de contaminations croisées sur une base toxicologique.
Publication of texts of consolidation on several topics, in particular as regards biosimilar products and the assessment of the risks of cross-contamination on a toxicological basis.

Origine	Titre	Type	Date
FDA	Considerations in Demonstrating Interchangeability With a Reference Product *Définition des principaux éléments à fournir afin d'établir l'interchangeabilité du biosimilaire au regard du produit de référence.*	Draft	17/01/2017
EMA	Questions and answers on implementation of risk based prevention of cross contamination in production and 'Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities' (EMA/CHMP/CVMP/SWP/169430/2012) *Question & réponses relatives à l'implémentation des risques de contaminations croisées évalués sur une base toxicologique.*	Q&A	15/12/2016
EMA	Guideline on process validation for finished products - information and data to be provided in regulatory submissions *Révision technique sans modification des concepts*	Révision	21/11/2016
WHO	Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products *Critères de démonstration de la similarité entre le biosimilaire (SBP) et le produit de référence (RBP).*	Draft	21/10/2016
ICH	Q3C(R6) – Impurities: Guideline for residual solvents. *Introduction des PDE pour la triéthylamine et la méthylisobutylcétone.*	Step 4	20/10/2016

Analytique - Analytical

L'USP poursuit sa démarche volontariste en matière de AQbD.
USP continues its deliberate effort in terms of Analytical Quality by Design.

Origine	Titre	Type	Date
USP	Stimuli to the Revision Process: Proposed New USP General Chapter: The Analytical Procedure Lifecycle <1220> *Introduction des concepts du QbD au développement des méthodes analytiques.*	Draft	17/10/2016

Réglementaire

By AKTEHOM

Fabrication - Manufacturing

Sur la période, outre les révisions de BPF et les modalités spécifiquement US, le guide FDA relatif aux produits combinés va permettre une meilleure compréhension des attendus sur cette thématique.
On the period, in addition to the revision of French GMP and specific US terms, the FDA guideline on combined products will give a better understanding of the expected results in this area.

Origine	Titre	Type	Date
FDA	Mixing, Diluting, or Repackaging Biological Products Outside the Scope of an Approved Biologics License Application *Cadre de tolérance pour le mélange, la dilution et le reconditionnement de produits biologiques hors des critères définis du BLA.*	Draft	12/01/2017
FDA	Repackaging of Certain Human Drug Products by Pharmacies and Outsourcing Facilities *Cadre de tolérance pour le reconditionnement de produits pharmaceutiques par des tiers autorisés.*	Final	12/01/2017
FDA	Current Good Manufacturing Practice Requirements for Combination Products *Modalités d'application des cGMP aux produits combinés dans l'objectif de clarifier et d'expliquer les attendus.*	Final	11/01/2017
FDA	Drug Supply Chain Security Act Implementation: Identification of Suspect Product and Notification *Identification et notification des produits suspects par l'ensemble des intervenants à la supply chain.*	Final	08/12/2016
ANSM	Révision annexe 15 : Qualification & Validation et annexe 16 : Certification par une personne qualifiée et libération des lots *Intégration en droit national de l'annexe équivalente de EU GMP*		06/01/2017
ANSM	Introduction en partie III des lignes directrices du 19 mars 2015 relatives à l'évaluation formalisée du risque visant à déterminer les bonnes pratiques de fabrication appropriées pour les excipients utilisés dans les médicaments à usage humain *Intégration des lignes directrices EU 2015/C 95/02*		30/12/2016

Système Qualité – Quality system

La FDA introduit le nouveau concept de Quality Metrics et formalise les attendus dans le cadre de la sous-traitance d'activités GMP.
The FDA introduces the concept of Quality Metrics and provides the requirements in the framework of outsourcing of GMP activities.

Origine	Titre	Type	Date
FDA	Submission of Quality Metrics Data *Définition du processus de communication à la FDA des mesures réalisées pour surveiller les systèmes de contrôle qualité et le procédé de fabrication.*	Draft	23/11/2016
FDA	Contract Manufacturing Arrangements for Drugs: Quality Agreements *Requis de définition et documentation applicables aux parties impliquées dans des activités de fabrication cGMP contractuelles.*	Final	22/11/2016

Steridico

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

T comme...

Taux, Teneur Concentration et Gaz Non Condensables

Chers amis lecteurs,

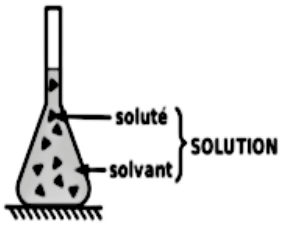
Toujours **T**rès **C**ritiques et **T**outes **T**endances **C**onfondues comme nos ambitions de qualité, la rigueur de l'usage des mots traduit notre **T**olérance **T**imide ou **C**ourageuse. Osons encore quelques précisions pour tendre vers la justesse.

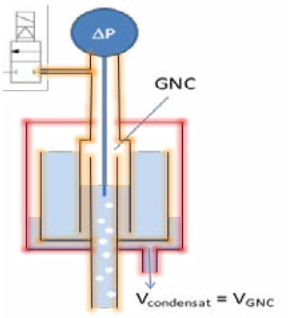
Alors pour le **Taux** (rate), si personne ne remet en cause sa définition, ce rapport quantitatif entre deux valeurs de même unité, on pourrait en douter par l'usage de notre langue. Celui-ci est donc exprimé sans unité, le plus souvent en pourcentage ou ses sous-multiples : ***‰, ppm, ppb***, ...
Pourtant dès le 14ème siècle, le vieux "françois" utilisait déjà le taux en synonyme de montant, prix ou somme et parfois dans son expression féminine : la fameuse "taxe".
Chacun s'interpellera sur ses expressions préférées. En effet 2,… enfants par famille n'est pas un taux de natalité mais une moyenne car le taux lui est exprimé, en ‰ , par le nombre de naissance annuelle rapportée à la population totale. Mention spéciale au "taux d'alcoolémie" puisque l'alcoolémie n'a pas de taux et est elle-même par définition une concentration d'alcool (pur) dans le sang exprimée en g/L.

> *NB : Le **titre** pour la vapeur (différent de sa définition en chimie) thermiquement est aussi un taux puisqu'il quantifie la masse d'eau liquide dans la vapeur rapportée à la masse totale du mélange vapeur + eau ou le rapport des chaleurs latentes : mesurée / théorique, aux conditions définies.*

La **Concentration**, qu'elle soit massique, molaire ou autre, exprime par des valeurs quantitatives le sens premier de l'action de concentrer (étymologiquement "avec au centre" = regrouper). De nombreuses industries, du textile à la chimie, de l'agro-alimentaire à la pharmacie bien sûr, emploient cette variable dans leur recherche et leurs procédés. Avec cette définition pour une solution, la masse d'un corps dissous (soluté) rapportée au volume de son solvant est généralement en g/L. On évoquera donc bien à juste titre une concentration de chrome dans un acier inoxydable ou de chlore dans l'eau mais on évitera la synonymie de biocharge et concentration en microorganismes.

⇢





Alors concentrons-nous sur un détail : quid de la concentration de **G**az **N**on **C**ondensables* (**GNC**) dans la vapeur d'eau ? Que l'on calcule en masse ou en volume *(cf Fig.1)*, le résultat étant un rapport sans unité, on l'exprimera comme un taux.

Comparison of rate calculations mass or volume of Non-Condensable Gases in the steam when using the sampling method described in EN 285.								
Dominique WEILL avec l'exclusive autorisation du CEN/TC102/WG3 Feb. 2014					exemple : sterilizer inlet @ 3 bar			
Steam pressure to be tested (kPa)	A	204,9	300	350	400	450	500	600
Steam temperature to be tested (°C)	B	121,11	133,5	138,87	143,63	147,92	151,85	158,84
Atmospheric pressure to collect NCG and condensate (kPa)	C	101,325	101,325	101,325	101,325	101,325	101,325	101,325
Ambiante temperature to collect NCG and condensate (°C)	D	20	20	20	20	20	20	20
Volume of collected water (mL) @ 101,325 kPa / 20°C	E	1000	1000	1000	1000	1000	1000	1000
Volume of collected NCG (mL) @ 101,325 kPa / 20°C	F	35	35	35	35	35	35	35
Calculation in mass								
Volume of condensate from steam (mL) @ 101,325 kPa / 20°C (E-F)	G	1000	1000	1000	1000	1000	1000	1000
Mass of condensate (g) @ 101,325 kPa / 20°C	H	998,3	998,3	998,3	998,3	998,3	998,3	998,3
Density of NCG (g/L) @ 101,325 kPa / 20°C /100% HR (similar to moist air)	I	1,194	1,194	1,194	1,194	1,194	1,194	1,194
Mass of NCG (g) (F x I / 1000)	J	0,0418	0,0418	0,0418	0,0418	0,0418	0,0418	0,0418
NCG / Steam rate (mass) (J x100 / H) (%)	K	0,00419	0,00419	0,00419	0,00419	0,00419	0,00419	0,00419
For information ppm		*41,9*	*41,9*	*41,9*	*41,9*	*41,9*	*41,9*	*41,9*
Calculation in volume								
Density of saturated steam (g/L) @ pressure and temperature of test	L	1,162	1,651	1,908	2,163	2,417	2,669	3,17
Volume of steam (L) @ pressure and temperature of test (H / L)	M	859,1	604,7	523,2	461,5	413	374	314,9
Volume of collected NCG (mL) @ pressure and temperature of test	N	23,28	16,4	14,24	12,6	11,32	10,28	8,71
NCG / Steam rate (volume) ($B_{saturé}$ / A x C / ($D_{saturé}$ x F) (N / (1000 M+N)) (%)	O	0,00271	0,00271	0,002722	0,00273	0,00274084	0,002749	0,00277
For information ppm		*27,1*	*27,1*	*27,2*	*27,3*	*27,4*	*27,5*	*27,7*

Fig 1 : Comparaison des calculs de taux de GNC en masse et volume dans la vapeur lorsqu'on utilise la méthode d'échantillonnage décrite dans l'EN 285 (2016).

Mais on portera la plus grande attention à l'expression des résultats issus de la méthode d'essai proposée par la norme EN 285 (2016) qui révèle un volume de gaz de **GNC** obtenu à partir de la collecte d'un volume de condensats liquides. Le seuil maximal est de 3,5 ml de gaz pour 100 ml de condensat et non ≤ 3,5%. Ce n'est donc ni une concentration ni un taux et bien une teneur (content) telle que correctement décrite dans la dernière révision. Les termes "concentration" et "%" ont été bannis.

*NB : * Les gaz dits "non condensables" ne sont pas "incondensables" puisqu'ils étaient dissous dans l'eau d'alimentation du générateur de vapeur. Ils ont été dissous dans des conditions géologiques complexes bien différentes et non reproductibles aux conditions du procédé d'exploitation de la vapeur pharmaceutique.*

Pour être complet, la **teneur** en chimie comme au sens large, traduit ce qui est contenu dans une substance, une solution, un rapport, un gâteau ou une note de musique. En chimie, c'est la quantité d'un composé chimique principal ou accessoire parmi d'autres. Par exemple, la teneur en eau dans une sache de 2 kg de bouchons peut être de 6,5 g mais le taux d'humidité résiduelle (TxHR) sera 6,5/2000= 3,25‰. Le taux de siccité exprimant l'état sec de la charge n'est pas l'inverse du taux d'humidité résiduelle mais son complément soit 1-(Tx HR).

Confidence d'une goutte d'eau concentrée sur son avenir : "La teneur de mon titre s'ennoblit, même sans particule, dès l'apparition de mes vapeurs".

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SGS Life Science Services est reconnu pour son expérience analytique dans le développement, le transfert et la validation de méthodes ainsi que le contrôle de la qualité des médicaments et de ses matières premières.

D'une surface totale de 2 100 m², situé à proximité immédiate des principaux hubs logistiques, notre nouveau laboratoire BPF situé à Villeneuve-la-Garenne (92) a pour objectif de réduire le délai de traitement analytique des échantillons grâce à une efficacité technologique et opérationnelle accrue, mais aussi de soutenir scientifiquement ses clients dans la production et le développement des produits biologiques. Avec un réseau international de 20 laboratoires de contrôle qualité, de bioanalyse, de sécurité virale et de caractérisation cellulaire situé dans 11 pays, SGS, votre partenaire Santé-Qualité, vous propose des solutions performantes personnalisées.

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ACTUALITÉ

31 mai & 1 juin 2017
Congrès A3P Bioproduction

BIOPRODUCTION EFFICIENCY
Process / Facilities / Equipment / Regulatory

10 lectures, 5 workshops, 1 exhibition and 3 plant visits.
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 forum. 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.

Bilan 2016

244 participants

12 conférences
10 ateliers
3 visites de site
32 exposants
136 sociétés présentes
14 pays représentés

Satisfaction

3,3 /4*
94% des répondants sont satisfaits à très satisfaits

« Very good atmosphere, easy contact »

« Good representativ of attendee (quality, R&D, production...) »

Info & Inscription www.a3p.org

BioProduction International congress

CITÉ INTERNATIONALE
50, quai Charles de Gaulle // Lyon (France)

10 Lectures

Conferences Simultaneous Translation

WHO GMP for biological products – major learnings
Mustapha CHAFAI, **WHO**

A pro-efficient methodology for rapid scale-up of mAbs Upstream Processing to Manufacturing
Abdel ZEMMAR, **LFB BIOMANUFACTURING**

#The challenges of developing a high concentration monoclonal antibody product
Antoine ALARCON, **SANOFI**

Bioformulation for the future: new alternative approaches are emerging, based on gained experience over the last 20 years in developing monoclonal antibody biotherapeutics
Otmane BOUSSIF, **NOVARTIS**

Biocontamination Body of Knowledge (BooK) for Drug Substance processes: turning lessons learned into action !
Guillermo José ALBANESI, **SANOFI PASTEUR**

Management of Economics in Biopharma Production, Opportunities, Reality & Myths
Gunther JAGSCHIES, **GE**

Flexible & cost effective GMP biotech facility: Testimony after 5 years' experience
Jérôme PIONCHON, **MERCK BIODEV**

Increase Bioproduction efficiency and safety with the addition of an integrity testing step
Fabien DEBRAS, **ZOETIS**

Clarifying the gap between upstream and downstream - Application of technologies to clarification of harvested mammalian cell culture
Christopher WILSON, **ALLERGAN BIOLOGICS**

Integrating Human Performance Principles into BioPharma Operations
Michael MODLER, **LONZA**

Exhibition

Sociétés exposantes déja inscrites : **3M Purification, Acm Pharma, Beckman Coulter, Biomerieux, Bioquell, Boccard Process Solutions, Burkert Contromatic, Cellon, Confarma, Eurofins Biopharma, Hamilton, Indatech, Meissner Filtration Products, Merck Life Science, Mettler Toledo Analyse Industrielle, Optima Pharma, Pall Life Sciences, Pharmasep, Resultance Life, Sartorius Stedim Fmt, Sgs Life Science Services, Spectrum, Steris, System C Industrie, Telstar, Thermo Fisher Scientific, Verdot Ips2, ...**

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BioProduction international congress

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5 Workshops

W1 # Implementation and validation of a single-use mixing step for virus clearance

LFB & SARTORIUS

LFB has recently invested in additional production capacity and is establishing a single-use production platform for the manufacture of monoclonal antibodies including a virus inactivation step. LFB chose single-use mixing bags over stainless steel tanks because they operate a multi-product facility using product-dedicated virus inactivation vessels. Using single-use mixers therefore decreases the investment required through having to purchase and validate a cleaning procedure for multiple stainless steel mixers. Numerous testing (chemical compatibility and leachable studies, film adsorption, mixing homogeneity) have been performed to demonstrate the applicability of single-use mixing technology for a virus inactivation step.

W2 # How to increase the quality without affecting the process validation ? Case study : cell culture automation from embryos

GSK & WOW TECHNOLOGY

In this workshop, you will experience how to manage the innovation in your sector by improving the quality without any compromise on the validation of the process. Our case study, based on the automation of the cell culture from embryos and used in MMR vaccine production, will give you the main guidelines to move forward and directly apply them to your specific projects.

W3 # Evaluation and decision criteria for Single Use Implementation in Bioproduction

VIRBAC & THERMOFISHER

Single-Use Systems (SUS) are significantly developing in order to meet the industry needs and requests. The advantages are well known : ease of use and implementation, decrease in cleaning validation effort , flexibility, limited CAPEX as compared to conventional systems....Nevertheless, many criteria need to be evaluated before implementing SUS in order to ensure success. During this workshop, we will invite you to take the role of a project manager in order to map the different steps and criteria for the implementation of a single use process. In a second phase, we will select 2 important topics and explore them in details. The goal of the workshop is to give participants an effective approach in the assessment of Single Use System projects.

W4 # Risk analysis in industrial biotech process development, robustness and consistency production run

DEBIOPHARM & ABCONSULTING

In the pharmaceutical industry, risk management has become a routine approach to the evaluation of unexpected events affecting quality, safety and performance of products and manufacturing processes ensuring, ultimately, optimal protection of the patient. Regulators expect that risk management is inherently built into the quality management systems of the pharmaceutical companies by using both formal and informal risk tools (see ICH Q8-9-10). This workshop will aim to first and quickly review the different guidelines, definitons and possible tools. Then, the key principles will be applied in practical case studies in small size working groups. In these case studies, the participants will be faced with real-life management of risks in different business scenarios and will be asked to analyse the situation and propose a sound risk management strategy and execution. The last part of the workshop will be devoted to sharing the outcomes of the different groups and comment the proposed solutions.

W5 # Stabilization in solution of the large molecules from the development to the fill & finish

UNITHER PHARMA & DISPOSABLE LAB

More than 50% of the new drugs coming from the biotech are nonstable in solution and require a freeze-drying form. Some technologies are available on the market and some new are under development.
The goal of this workshop is the following: - Describe the different technology applied to stabilize in solution some large molecules - The application for Drug substance (DS) and Drug product (DP). Biodisponibility, pharmaceutical form, side effects....- Compare a process and steps for a freeze dry form and a liquid injectable form for a small batch and what are the advantages and disadvantages. Sterilization, handling, formulation, cost...- The fill&finish solution for small batches as disposable Isolator, RTU materials (Vials, stopper....) and the regulatory rules- In term of filling solution what are the technology to fill small volume as its required in many gene therapy field (0, 1 or 0, 2 mL)?
The workshop will be performed around a Process Chart which define the steps and choices using the experience of the participant and based on 2 case studies from the DS to the DP including technology, safety, cost... Freeze dry form - Liquid form.
This Workshop will attend people from the development, formulation and clinical phases.

Info & inscription www.a3p.org

BioProduction International congress

CITÉ INTERNATIONALE
50, quai Charles de Gaulle // Lyon (France)

3 Plant visits

ACCINOV

Accinov is a biomanufacturing centre and provides biotech, pharma and medtech companies with state of the art R&D laboratories (BSL2), cleanrooms (ISO 8-7), GMP manufacturing pilot units & tailored solutions for quality assurance & pharmaceutical support to boost their projects, keep internally their know-how and their IP & manage their facilities in the most flexible way. Accinov's customers have just to plug their projects & play in a full ISO/GMP compliant environment. Accinov has had pharmaceutical establishment status since September 2014 and hosts more than 80 person on site, 15 companies and projects, including experimental new drugs production projects.

The afternoon will start with a presentation of the Do-it-yourself GMP manufacturing model for biologics manufacturing through a real case study of an experimental drug developed at Accinov. After the presentation, Acinov's team will invite you for a site tour to discover the GMP pilot units dedicated to cell banks, biological drug substances & non-sterile & sterile drugs production. During the visit, you will also learn more about the innovative projects hosted in the biomanufacturing center.

BIOMERIEUX

Craponne is bioMérieux major site for culture media manufacturing and the largest in Europe: more than 130 million Petri dishes and 20 million tubes & bottles are produced every year, the site also provides ready-to-use Deshydrated Culture Media to the other manufacturing sites.

Referring to the pharmaceutical business, 30 Millions of plates are produced in Craponne. The site is indeed the place where the 3P range of pharmaceutical culture media for environmental monitoring was developed and is currently produced, with the highest standard available in the field of culture media. Considering this core part of our activity, the brand new pharma-dedicated area, Petri 3, launched in 2015 allowed us to double our 3P range manufacturing capacity.

The visit will be organized around 4 complementary workshops:

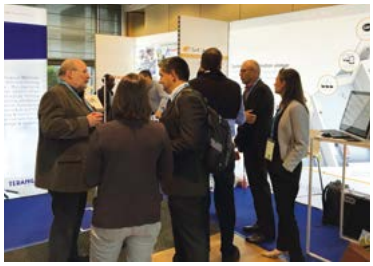
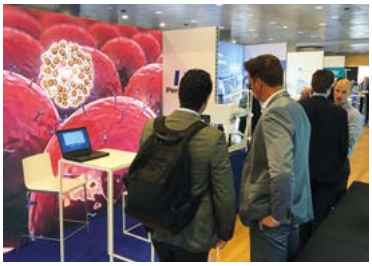
- *Site global presentation (classroom)*
- *Petri 3 area visit (remote controlled high resolution camera system)*
- *Tubes & Bottles area visit (remote controlled camera system)*
- *Quality Control lab visit (on the shop floor)*

SANOFI GENZYME

This Sanofi Genzyme manufacturing site, based in the Biodistrict of Gerland, is specialized in bioproduction and immunology. It is also a reference in terms of environmental protection. The Lyon site manufactures, for 68 countries, polyclonal antibodies used to prevent and treat graft rejection during transplants and in hematology. The team is in charge of the management of all bio-materials, purification of the immune serum and the pharmaceutical release of the active ingredient.

Since 2015, the site diversified its activity with the implementation of a viral vector manufacturing platform which represented an investment of 10 million euros, financed equally by Sanofi and Transgene (partnership signed in 2013). This platform will enable production of a new therapeutic class of active ingredients (viral vectors) and will be made available to outside customers as well as for internal needs.

During the visit, you will have a presentation of the site activities and get to visit our premises, including both manufacturing platforms.

Info & inscription www.a3p.org

ACTUALITÉ

4th May 2017, Novotel Bern
ICHQ3D Elemental Impurities

8 months before the go live. Are you ready for the implementation?
The guidance is applicable since June 2016 for New products and will be effectiv ein December 2017 for all the products.
During the day, Risk analysis to manage the control of elemental impurities in the product will be presented integrating the products / process knowledge. Shop floors experiences will be also detailed to illustrate the different approaches which can be applied to be in compliance with this new guidance.

Operational Implementation of ICH Q3D - Proposed Analytical Methods :
Elise GALLAIS - SGS

•

Implementation Strategy of ICH Q3D guideline for a parenteral long term sustained release formulation : Marco SALVAGNI - DEBIOPHARM

•

Feedback from a Biotech industry on ICH Q3D Implementation : Aurélie DUCLOS - VIFOR PHARMA

•

How to implement ICH Q3D - Defining a Risk-Assessment Strategy for Elemental Impurities : Elisabeth MOREAU - LABORATOIRES BOUCHARA-RECORDATI

•

ICH Q3D - Implementation, a control strategy in Biotech - Feedback of the "drug product" solution used to monitor Elemental Impurities based of the Risk Assessment approach : Sylvain PLAGELLAT - MERCK

•

Elemental Impurities testimony of implementation by a contract manufacturing organization : Jean-Marc LIBERSA - FAREVA

•

PLANT VISIT OF CSL BEHRING

Info & Inscription www.a3p.org

ACTUALITÉS

4 & 5 juillet à Lyon

Journées A3P CDMO

- Stratégie de la sous-traitance
- Risques en CDMO
- Sous-traitance analytique - fabrication - distribution
- Retour d'expériences
- Enjeux sociaux

14 Conférences / 4 Ateliers / 1 Table ronde / 1 Exposition

A3P CDMO Days

L'Association A3P a, depuis 30 ans, la vocation de mettre en commun les expériences des utilisateurs et des fournisseurs de l'industrie pharmaceutique / bioproduction. Les 4 et 5 juillet 2017 à L'Espace Tête D'Or à Lyon, A3P organise les journées CDMO (Contract Development and Manufacturing Organization). Durant ces deux jours, 150 professionnels participeront aux 12 conférences et aux 5 ateliers proposés.

Un CDMO intervient à des degrés divers sur l'ensemble des activités des groupes pharmaceutiques. Ces journées proposent donc aux participants d'apporter sur ce sujet d'actualité, les meilleures expériences dans le domaine complexe qu'est la sous-traitance.

Les thèmes abordés et développés lors de ces journées seront les suivants :

• Stratégie de la sous-traitance : stratégie industrielle et risques
• Les risques en CDMO
• Sous-traitance de produits atypiques : cytotoxiques, produits vivants, hormones, …
• Sous-traitance analytique
• Sous-traitance de fabrication : essai clinique lots phases 1,2,3
• Sous-traitance distribution/activités annexes : transports, chaîne de froid, stockage, …
• Changement de big pharma à sous-traitance
• Retour d'expériences
• Enjeux sociaux, changement de culture : cession de sites

Le programme de 2 jours a été conçu pour offrir un bon équilibre entre les conférences délivrées par des leaders d'opinion, des ateliers animés par un tandem d'utilisateurs / fournisseurs et une exposition de prestataires ; le tout dans une ambiance propice aux échanges !

Info & inscription www.a3p.org

14 Conférences

Conferences Simultaneous Translation

- Outsourcing et sous-traitance : stratégie industrielle, challenges et risques associes
Sonia KASPEROWICZ - *GSK &* **Jacques Navellou** - *Axys Network*
- Responsabilités du donneur d'ordre avec un cas concret de sous-traitance d'un vaccin
Pierre LAUER - *Sanofi Pasteur*
- Strategy for the development of an innovative parental drug product containing two MABs. Keys to success in a outsourced challenging project
Alain SAINSOT - *Amatsi Group*
- Sous-traitance d'analyses, face à un marché à forte croissance, le développement d'un site. Retour d'expérience d'un transfert de site, 800 personnes, 10000m² réalisé en 6 semaines
Adrien PUISSEGUR *&* **Yassine BOUHID** - *Groupe Carso*
- L'excellence opérationnelle et la conformité réglementaire : 2 objectifs clés
Marc MAURY - *Unither Pharmaceutical &* **Paul BEYOU** - *SGS*
- Maîtrise de la qualité des producteurs chez les sous-traitants par le biais du contrat Qualité établit selon la FDA guidance de novembre 2016
Nathalie PARISOT - *Celgene*
- Production d'IMPs - Retour d'expériences d'un promoteur non pharmaceutique
Marianne PERDRIJAT - *DBV &* **Nicolas LE RUDULIER** - *Creapharm*
- Sous-traitance : Quel niveau de suivi ?
Jihaine NAJAH-BAUD *&* **David DA CUNHA** - *Roche*
- Recovering the GMP certification after the acquisition of a manufacturing site by a CMO
Rovi Contract Manufacturing
- Du Grand Groupe au CMO pour un voyage sans embûche
Jean-Yves SARCIAUX *&* **Thibault DEMOULIN** - *Laboratoires Macors*
- Le rôle du dépositaire dans l'industrie pharmaceutique
Frédérique ROUQUÈS - *Alloga*
- Un service Qualité allié à des packagings de qualité, le tout géré dans un réseau en propre pour un maintien de la chaîne du froid optimal
Sébastien PRADAL - *World Courier*
- Enjeux et challenges de la création d'une BU CDMO au sein de Servier
Gwenaël SERVANT - *Servier CDMO*
- Réussir une cession de site industriel : Facteurs clés de succès/Retour d'expériences
Ludovic MATHIEU - *In Altum Consulting &* **Roland BLONDEL**

4 Ateliers

- *Atelier 1 Contrat de fabrication/contrat de façonnage : quelle différence?*
Emmanuelle DE SCHEPPER *&* **Jean-Marc GROSPERRIN** - *Dentons*
- *Atelier 2 La gestion de crise dans le cadre d'une production chez un CMO*
Julien TRIQUET - *GSK &* **Patrick ROUSSEAU** - *Aspen*
- *Atelier 3 Comment intégrer nos clients dans le programme de stérilisation ?*
Recipharm
- *Atelier 4 Important BFS CMO Aspects*
Martin HAERER & Christoph BOHN - *Rommelag CMO*

Info & Inscription www.a3p.org

Frontière Part I/ Part II des BPF : Modalités d'application aux produits biologiques.

Par Leem - Groupe de travail "Bioproduction/Qualité/Réglementaire"

Suite aux questionnements des Entreprises Françaises de Biotechnologie sur la segmentation proposée par les inspecteurs des parties I et II des BPF vis-à-vis du procédé de production des médicaments biologiques, cet article a pour objectif de proposer une clarification argumentée du cas particulier de ces substances actives biologiques dont la formulation est réalisée lors de la production de substance active et non au moment de la fabrication du produit fini. Ce principe s'applique pleinement aux protéines recombinantes et anticorps monoclonaux, et au cas par cas des procédés de fabrication, pour d'autres types de médicaments de biotechnologie et thérapeutiques innovantes (vecteurs de thérapie génique et vaccins, …).

Problématique

Le contexte

Lors de la production d'un composé chimique, les étapes de synthèse de la substance active (DS) et de mise sous forme pharmaceutique du produit fini (DP) sont séparées dans le temps et souvent géographiquement.

Lors de la production d'une substance biologique, et plus particulièrement pendant sa purification, il est indispensable dans la majorité des cas d'ajouter instantanément des agents permettant sa stabilisation. En effet, une substance biologique n'étant pas stable, elle risquerait de se dénaturer et ainsi de perdre sa conformation native ou de subir très rapidement des dégradations. La dégradation de la protéine d'intérêt ou sa dénaturation conduirait à une perte de son efficacité thérapeutique ou à un risque d'immunogénicité.

La problématique discutée dans ce document consiste par conséquent à proposer une clarification des limites d'application des deux parties du référentiel réglementairement opposable pour :

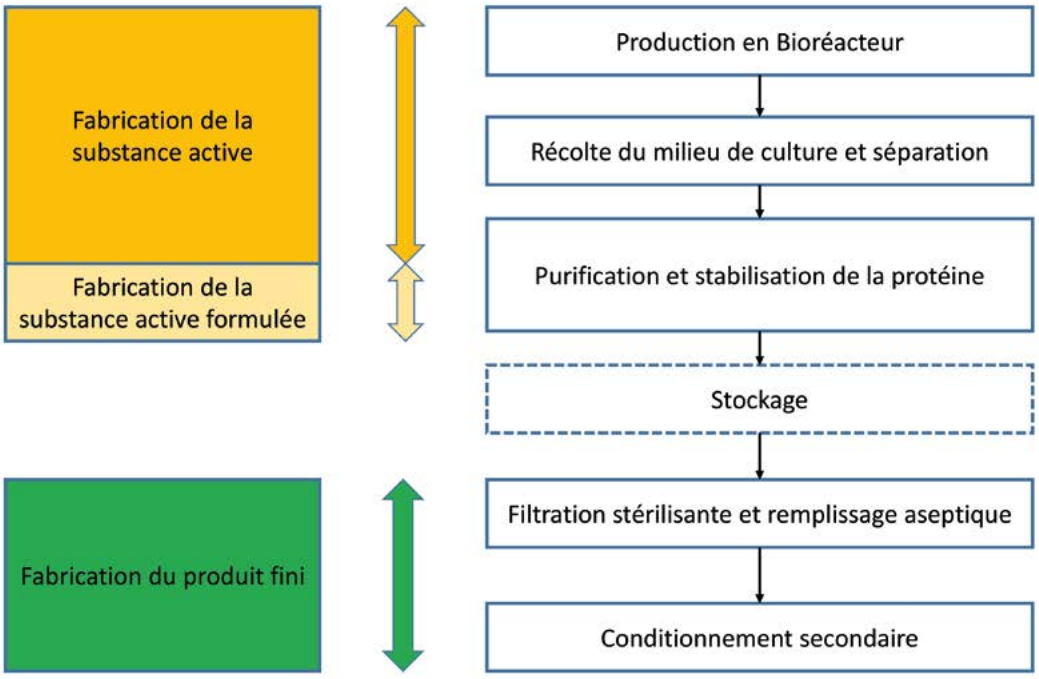
- la fabrication et le conditionnement sous forme vrac de la substance active biologique, d'une part,
- la fabrication du médicament biologique à partir du vrac de la substance active jusqu'à son conditionnement final, d'autre part.

⇢

De ce fait, les modalités d'application de ce référentiel ont des conséquences sur le statut réglementaire en France du site fabricant.

La chaîne de fabrication d'un produit biologique

La particularité d'une chaîne de fabrication de produit biologique réside dans la nécessité de réaliser une formulation de la substance active au cours des étapes de purification ou d'obtenir un vrac stabilisé pouvant être stocké.
Ainsi, c'est la plupart du temps une substance active formulée qui est libérée par le site de production de la substance active.



Répartition des étapes de fabrication au sein d'un procédé de produit biologique dans lequel la formulation est réalisée en cours de purification

La formulation des substances actives biologiques

La formulation d'une substance active biologique, telle qu'un anticorps monoclonal par exemple, consiste principalement en sa stabilisation.

La substance biologique étant vulnérable aux facteurs extérieurs, l'étape de formulation est donc réalisée dès sa purification, par l'inclusion des stabilisants dans les tampons de purification.

L'ajout des composants en fin de purification est nécessaire afin de stabiliser l'anticorps ou la protéine thérapeutique peu de temps après son extraction.
Il s'agit également de réduire au maximum les étapes de congélation/décongélation et de stockage du produit, afin de diminuer le risque de dénaturation du produit et de limiter les stades de "Produit Intermédiaire".

De ce fait, il est fréquent que cette stabilisation en cours de procédé soit la dernière étape de fabrication du produit avant la filtration stérilisante et la répartition du médicament.
Le produit qui est alors libéré par le fabricant est une substance active formulée, et non pas une substance active pure, comme dans le cas des produits chimiques. Cet état des lieux pose la question du niveau de segmentation entre la Partie I et la Partie II des BPF.

⇢

Discussion
Partie I et Partie II des BPF

Dans ce contexte des produits biologiques, il est nécessaire de repréciser le champ d'application de chaque partie de ce référentiel, sachant que l'annexe 2 des BPF couvre l'ensemble du processus de fabrication de ces médicaments biologiques et le niveau d'exigence BPF associé.
En France, la réglementation stipule clairement que l'application de la Partie I implique la notion d'établissement pharmaceutique fabricant.
L'application des BPF Partie II à des stades plus ou moins précoces de production de la substance active n'implique pas d'être établissement pharmaceutique.
La recommandation du groupe Bioproduction du Leem est résumée sur le schéma suivant qui compare les étapes de fabrication d'un médicament biologique à celles d'un médicament chimique et propose le niveau de segmentation entre les deux parties des BPF pour les deux types de produits.

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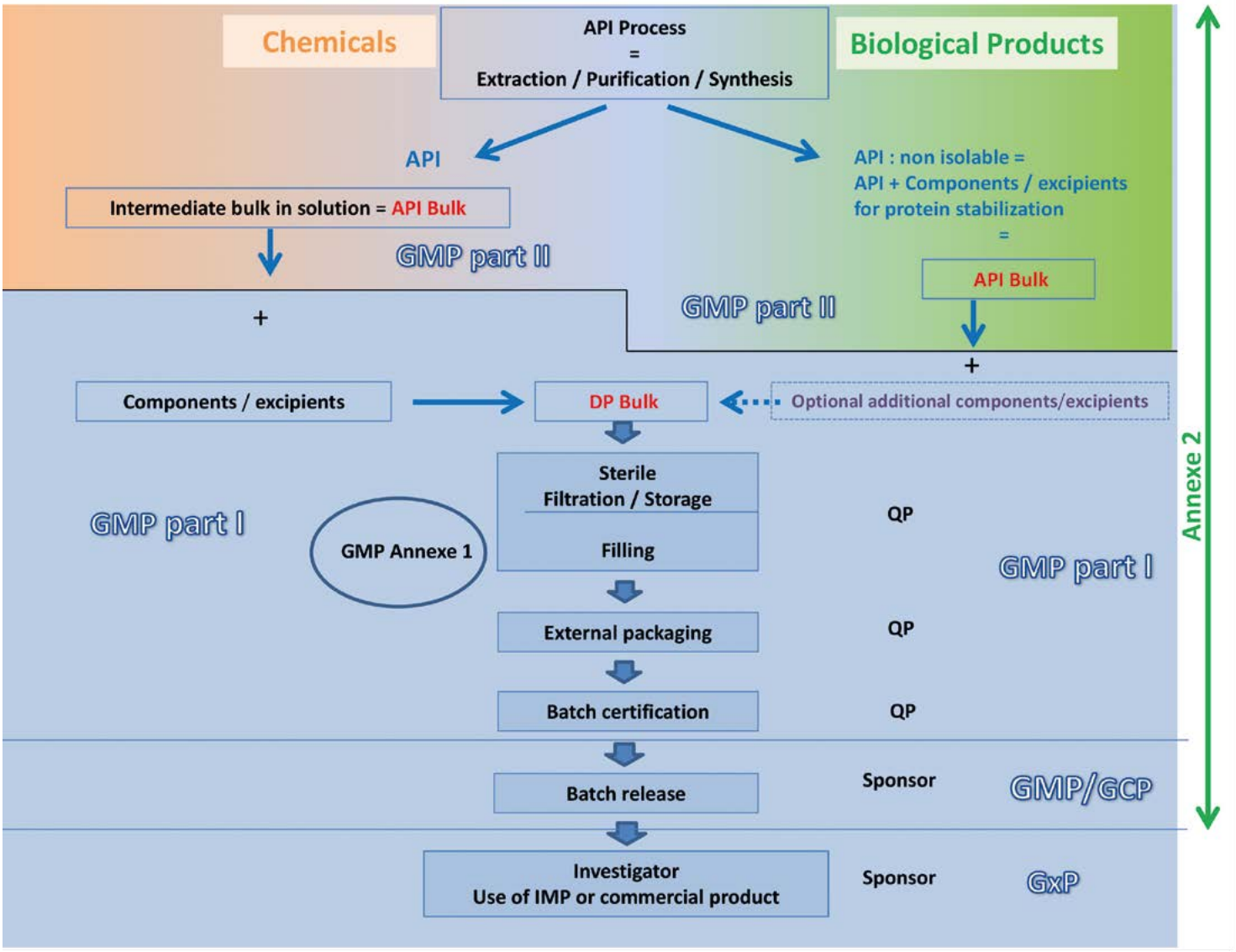

Répartition des étapes de fabrication d'un procédé de produit biologique et frontières entre Partie I et Partie II des BPF/ GMP

Notre lecture des BPF est basée sur les points suivants :

- Les excipients sont nécessaires pour la stabilisation de la protéine.
- Les excipients sont généralement indissociables du procédé de fabrication de la substance active.
- Le statut de la substance active conditionnée en vrac est revendiqué "low bioburden" plutôt que "stérile" (même si une filtration 0.2µ est effectuée) car la filtration stérilisante est réalisée par la suite, dans les étapes de fabrication du produit fini.
- Il est également possible de faire la différence entre la substance active formulée et la fabrication du PF par la présence d'une étape de stockage dans le procédé, les étapes de stockage donnant lieu à des tests de stabilité et de validation du stockage stérile.
- La fabrication de la substance active jusqu'à son conditionnement est décrite dans la partie"Substance" du dossier réglementaire (soumis aux BPF Partie II) alors que la fabrication du médicament (Produit Fini / Drug Product) est décrite dans la partie "Produit" (soumis aux BPF Partie I). Ceci permet ainsi d'assurer la cohérence entre le dossier d'AMM et l'inspection.

Ainsi, comme mentionné dans l'annexe 2, l'application de la Partie I des BPF concerne le processus de fabrication du produit fini, à partir de la filtration stérilisante ou d'une étape de stockage, c'est-à-dire le remplissage aseptique dans sa totalité (filtration + répartition + intégrité des unités à injecter). Lorsque le procédé de fabrication d'un produit biologique ne comporte pas d'étape de stérilisation, la Partie I des BPF s'applique à des stades amont définis au cas par cas.

Le fabricant de substance active formulée dans le cas des produits biologiques, n'est donc pas dans l'obligation d'avoir le statut d'établissement pharmaceutique.

Remarque sur les contrôles de l'entité biologique : compte-tenu de la spécificité des étapes de fabrication d'un produit biologique, la substance active se trouve être généralement de même composition que le produit fini. Ceci peut justifier que les contrôles analytiques spécifiques du produit fini, nécessitant des équipements particuliers et une expertise, puissent être faits sur le site de fabrication de la substance active sans que celui-ci ne soit établissement pharmaceutique mais en tant que sous-traitant de l'établissement pharmaceutique libérant le médicament.

Conclusion

La règlementation française exige que les fabricants de principe actif pharmaceutique répondent à la demande d'autorisation d'activité de fabrication de substance active auprès de l'ANSM pour la ⇢

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Définitions

Substance active : Toute substance ou mélange de substances destinés à être utilisés pour la fabrication d'un médicament et qui, lorsqu'ils sont utilisés dans la production d'un médicament, devient un principe actif du médicament (. . .). *Equivalent : API, DS (Drug Substance)*.
Matière première : Terme général utilisé pour désigner les matières premières de départ, les réactifs et les solvants destinés à être utilisés dans la production des intermédiaires ou des substances actives.
Produit fini : Produit dans son conditionnement final en vue de sa libération. *Equivalent : DP (Drug Product)*
Produit intermédiaire : Produit partiellement manufacturé qui doit encore subir d'autres étapes de fabrication avant de devenir un produit vrac.
Produit vrac : Produit qui a subi toutes les étapes de la fabrication à l'exclusion du conditionnement final. *Equivalent : Bulk*

References

1. Extraits des Bonnes Pratiques de Fabrication, Bulletin officiel N° 2011/8 bis, publié en juillet 2011 par l'ANSM [59]
2. EudraLex - Volume 4 Good manufacturing practice (GMP) Guidelines: Part II - Basic Requirements for Active Substances used as Starting Materials; Part I - Basic Requirements for Medicinal Products;
3. Annex 1 Manufacture of Sterile Medicinal Products
4. Annex 2 Manufacture of Biological active substances and Medicinal Products for Human Use(171 KB)

production de produit vrac, sans que ceux-ci ne prennent le statut d'établissement pharmaceutique.
Autrement dit, il n'est pas nécessaire d'être établissement pharmaceutique pour fabriquer une substance active biologique formulée et les entreprises du médicament confirment qu'il est cohérent de ne revendiquer l'application de la Partie 1 des BPF qu'à partir de l'étape filtration stérilisante ou répartition aseptique, dans le cas où aucun composant/excipient additionnel n'est ajouté à la substance active stabilisée.
Toutefois, les fabricants concernés bénéficiant d'un statut d'établissement pharmaceutique sur toute la chaîne de production doivent pouvoir conserver ce statut, même s'ils se conforment à la télé-déclaration d'autorisation d'activité de fabrication de substance active auprès de l'ANSM.

Glossaire

BPF / GMP : Bonnes Pratiques de Fabrication / Good Manufacturing Practices
DS / API : Drug Substance / Active Pharmaceutical Ingredient ou Substance active
DP/PF : Drug Product / produit fini
AMM : Autorisation de Mise sur le Marché
ANSM : Agence Nationale de Sécurité du Médicament et des produits de santé
QP / PR : Qualified Person / Pharmacien Responsable
IMP : Investigational Medicinal Product

Moving One Unit Operation At a Time Toward Continuous Biomanufacturing.

By Peter Levison, PhD - Pall Life Sciences
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Many industries including those associated with generation of power and the manufacture of glass, steel and petrochemicals etc. have demonstrated that continuous manufacturing provides significant benefits and advantages, ranging from reduced capital and operating expenses to greater efficiency and product quality and consistency.

The traditional approach to biopharmaceutical manufacturing involves a sequence of batch unit operations separated by hold steps requiring additional tanks and/or biocontainers. Such an approach while effective is not entirely efficient since it fails to maximize facility use, requires large buffer volumes, and can result in overall inefficiencies and sub-optimal process economics.

An integrated, continuous approach to bioprocessing effectively connects each unit operation, minimizing the need for lengthy intermediate hold steps. Consequently, the overall process is more efficient than batch, occupies a smaller physical and environmental footprint (favorable process mass intensity, PMI, values and environmental factors), requires less initial capital investment and has lower overall operating costs. By extending the operating period (weeks or even months compared with hours to a few days) use of smaller scale equipment continuously can actually yield large quantities of product. In addition, the increased automation for continuous operations results in greater process control, more consistent product quality, minimal downtime and minimization of human intervention, reducing the likelihood of nonconformance and increased operator safety.

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Development times also are often shorter for continuous than for batch-based processes.
Continuous solutions from Pall Life Sciences, for both upstream and downstream biopharmaceutical unit operations, are enabling innovative biologic drug manufacturers, Contract Research Organizations (CROs), and Contract Manufacturing Organizations (CMOs) to maximize the benefits of continuous operations. Summarized below are the individual unit operations involved in the production of biologic drugs and the single-use, continuous solutions that are readily available or are currently being developed.

Biologic Drug Manufacturing

The biopharmaceutical production process begins with translation of the biologic drug in a bioreactor using a mammalian cell culture or microbial fermentation process. The major class of biologic drugs today are Monoclonal antibodies (mAbs) which are typically produced in mammalian cells, most frequently in Chinese hamster ovary (CHO) cells. Other recombinant protein therapeutics can be produced from mammalian cell lines, microbial and insect host cell lines.

Currently the majority of current upstream processes are based on fed-batch technology although the use of perfusion bioreactors is emerging rapidly. In the present article we concentrate on the downstream processing of recombinant proteins derived from fed-batch processes. For mAb production, CHO cells in cell culture media are cultured in the bioreactor (stainless steel or disposable) and fed necessary nutrients under conditions appropriate for maximum growth. The cell culture continues for several days (typically 10–17) to a specified end point. Having reached this set point, the harvested cell culture milieu contains the recombinant protein of interest along with many unwanted materials, including both live and dead (apoptotic) cells, cell debris, nucleic acids, host cell proteins secreted by the cell as byproducts and during cell death (apoptosis), and organelles and other types of contaminants.

Step1: Clarification

A biologic drug must first be separated from the particulate contaminants present in the cell culture prior to purification. This separation process is referred to as clarification and generates a clarified harvest cell culture fluid (HCCF). In a traditional process, the cell culture is passed through either a centrifuge or large-pore-size primary depth filter to remove a significant percentage of the solids and turbidity present. In each case there is typically a secondary polishing depth filtration step using a smaller-pore-size depth filter to achieve the target clarity needed to allow for bioburden reduction filtration prior to chromatography.
Pall secured an exclusive licensing agreement for acoustic wave separation technology from FloDesign Sonics in 2015 for continuous clarification of cell culture and introduced the Cadence Acoustic Separator (CAS) PD system in 2016. This technology has been shown to be scalable and currently a larger version of the PD system is being developed for use in cGMP manufacturing. CAS is a single-use clarification technology that enables continuous processing, which yields HCCF following polishing with a small depth filter suitable for subsequent chromatography.

Step2: Protein A Chromatography Capture

mAbs are almost exclusively captured using a Protein A chromatography step for primary purification. In this step, the mAb initially binds to the protein A sorbent while host cell proteins and other contaminants pass through. The column is then washed and bound mAb desorbed using a low pH buffer to elute the mAb.
Other recombinant protein biologics have different chromatographic purification requirements depending on their properties but it should be noted that Pall's continuous solutions are also applicable for those biologic drugs.
Continuous chromatography is achieved using the bench-top Cadence BioSMB PD system, the first multi-column chromatography system equipped with a single-use flowpath designed for biopharmaceutical manufacturing.
Based on countercurrent processing technology, the BioSMB technology can replace most batch chromatographic steps while maintaining existing sorbent chemistries and buffer systems. Because the columns are connected in series, the first can be loaded past product breakthrough since the second column will capture the breakthrough. The number of columns can be adjusted depending on the mAb titer of the HCCF and the desired relative flow rate. The single-use flow path provides flexibility in manufacturing configurations. Overall, the system provides improved productivity with a smaller footprint and reduced buffer tank requirements for overall cost savings since proportionally more of the column binding capacity is used compared to a batch column where loading must be terminated before product breakthrough.

Pall recommends the use of KANEKA KanCapA Protein A chromatography sorbent (from Kaneka Corporation) for the primary capture of monoclonal antibodies from clarified cell cultures. This sorbent has a high binding capacity to match current expression levels and is designed to provide high selectivity under mild conditions. It also exhibits good flow performance and alkali stability for reuse, making it ideal for continuous mAb capture processes.

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The Cadence BioSMB Process system is capable of processing HCCF from bioreactors as large as 2,000 L under cGMP conditions at flow rates of up to 350L/h. The Cadence BioSMB Process system also has a single-use flow path and leverages the design established for the PD unit.

Step3: Viral Inactivation

Following Protein A chromatography, the eluate containing the mAb is then subjected to a viral inactivation (VI) process involving treatment at low pH. This orthogonal step is conducted to ensure inactivation of enveloped viruses and typically is performed at around pH 4 for 60 minutes. It is possible to configure the Cadence BioSMB PD system to perform a VI step a continuous Cadence solution for this important downstream process that can be used in cGMP environments.

Step4: Ion-Exchange Polishing Chromatography

Following viral inactivation (and buffer exchange if necessary), the mAb is further purified using ion-exchange chromatography. This two-step process to remove host cell contaminants and possible antibody aggregates typically involves a bind-and-elute step using a cation-exchange sorbent followed by a flow-through step using anion-exchange. However, the order of the chromatographic steps may be alternated to accommodate process conditions.

As an example a polishing platform based on anion-exchange membrane chromatography using Mustang Q in flow through which directly loads onto a cation exchange mixed mode column containing CMM HyperCel for bind-an-elute purification. During this stage, remaining host cell proteins and other contaminants, including aggregates, are effectively removed. For continuous operation, both steps can be conducted using the Cadence BioSMB technology.

Final Steps: Preparing for Final Formulation

The eluate obtained after the ion-exchange polishing chromatography operations has the high purity and high quality required for final processing and formulation. Typically just four steps remain: virus removal filtration, buffer exchange (diafiltration), concentration, and sterilizing-grade filtration.

For mAbs, which are often formulated at high concentrations, diafiltration is typically performed before the final concentration step. A continuous in-line diafiltration (ILDF) to eliminate the traditionally recirculation TFF approach used for buffer exchange. By use of single pass TFF (SPTFF) technology ILDF can be accomplished in a continuous manner.

For continuous processes, biologic manufacturers can use the Cadence SPTFF technologies in various formats to achieve the target product concentration at high yield.

Continuous Development

Continuous biopharmaceutical manufacturing is being explored by both small and large drug manufacturers. These efforts have demonstrated successful continuous unit operations and integrated lab-scale continuous processes (upstream drug production to the final sterilizing-grade filtration step).

Although individual unit continuous operations can be applied today, there is still more work to do for a fully continuous process to be implemented in manufacturing. Further development of automation/control systems as well as in-line/at-line/on-line monitoring and analysis capabilities (process analytical technology) are needed to fully realize the benefits offered by continuous manufacturing.

Even so, the systems for biopharmaceutical manufacturers and the numerous technologies under development, support innovative companies that understand the value of continuous processing.

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The case study of BioMarin Fast-Track project with VERDOT Ips²

By Sébastien Lefebvre - Verdot IPS²
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Figure 1: VERDOT Ips² InPlace™ Chromatography Column

This paper reports a very fruitful collaboration between a Biopharmaceutical company, BioMarin International Ltd and a manufacturer of purification equipment, VERDOT Ips², which contributed to a fast start-up of an installation for manufacturing a recombinant human tripeptidyl peptidase 1 (rhTPP1), for the treatment of neuronal ceroid lipofuscinosis Type 2 (CLN2) disease, also known as Batten Disease.

The successful start-up of every manufacturing process depends on:

- Design and building of equipment, engineered for purpose and qualified to deliver the quality and efficacy attributes of the product;
- Validated procedures for operating, cleaning and maintaining operations, which must be optimized according to the installation;
- Trained and competent engineering and technical staff for operating, maintaining, and supporting the operation;
- Continued support by supplier-partners who maintain understanding of the equipment, application and project history through the qualification and start-up phases and hand-off to operations.

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Since 1987, VERDOT Ips² (www.verdotips2.com) is a worldwide supplier of purification solutions, especially in Low Pressure Liquid Chromatography, for applications in Biopharmaceutical and Nutraceutical manufacturing.

BioMarin (www.biomarin.com) is a world leader in developing and commercializing innovative biopharmaceuticals for rare diseases driven by genetic causes. In that respect, Children with CLN2 disease typically begin to present symptoms between the ages of two and four, with the majority of affected children losing their ability to walk and talk by approximately six years of age. During the later stages of the disease, feeding and tending to everyday needs become very difficult, and death often occurs between 8 and 12 years of age. Saving months on the market release of such therapy can save years of life expectancy for the young patients. Thus, after positive results obtained during clinical phase, BioMarin accepted to launch an early access program to provide experimental drug prior to marketing phase.
The collaboration between BioMarin Shanbally Ireland and VERDOT Ips² in this project started in September 2015. The goal was to start up a large scale process chromatography installation within eight months to respect the timing of the early access program.

In addition to the stringent planning, the project also involved difficult space constraints, as the new installation had to fit in existing production suite dimensioned for smaller scale. Smart chromatography column design, capable to reduce volume of consumables for its preparation (packing/unpacking/cleaning) was thus considered as a strong advantage for minimizing the impact on utilities, such as the WFI installation.

Excellent team work and communications was critical to responding to the challenge. The engineering teams of BioMarin and VERDOT Ips² worked closely together to address the space & utilities constraints with consideration of operational requirements and work ergonomics. The project managers of both companies met weekly through the project completion to satisfy the milestones of the planning, and respond to challenges in coordinating the supply chain of equipment and fabrication.

Standard Operating Procedures (SOPs) are the most important documents available to operators, engineers and maintenance technicians. They describe very precisely the implementation of the design intention with the installation, and ensure the consistency of quality and efficacy in batch, whatever the operator. Very often this document is drafted by a process expert alone, who writes it based on his own experience and following template procedures close to the ongoing application. The specificities of the installation and the current application can thus not be fully considered. This approach does not allow us to integrate new know-how from external experts, such as suppliers of consumables (chromatography resins, filtration membranes) or equipment suppliers. Useful tips can be found in the instruction manuals of each one, but these being generic are rarely applicable as described.

Based on successful experience between the two partners, **BioMarin and VERDOT Ips²** implemented an intensive collaborative approach to speed up the preparation for installation and commissioning of the equipment. This approach was based on the co-writing and co-validation of the SOPs. Following BioMarin's standard format and based on the existing installation and quality attributes defined for the new product, BioMarin and VERDOT Ips² very openly shared their experience to write the detailed procedures for equipment preparation: packing, unpacking and pre- and post -packing cleaning. The new procedures were pilot tested in generic and non-GMP conditions, just after the Site Acceptance Test. This early procedure walk-through allowed optimization of parameters settings and sequence of operations. The revised procedures were tested again in cGMP conditions with the set of buffers and stationery media defined for each purification step. The static phases, i.e.: chromatographic media, were supplied from various international suppliers. One also provided his assistance during the packing.

BioMarin and Verdot Ips² also know from experience that important design features can often be left behind in the hand-off of equipment from supplier to customer engineering to operations. Without critical communication, awareness, and training, operational practices quickly default to familiar techniques developed on obsolete equipment. The opportunity provided by a new manufacturing process with purpose-engineered equipment and an aggressive timeline means everyone involved can be hyper-focused on the details of change.

Results and Discussion

Column cleaning

The column was sanitized with 1N NaOH (sodium hydroxide), injected via suction using the motorized adapter as a large syringe. The adapter equipped with an inflatable seal was fully soaked in Sodium hydroxide with seal deflated. After 2 hours of soaking, an alternated circulation of NaOH between down flow and up flow performed a full sanitization of the distributors and bed support. The design of the adapter ensured that the mobile phase was distributed everywhere, enclosing the border of the dynamic seal to avoid risk of bioburden.

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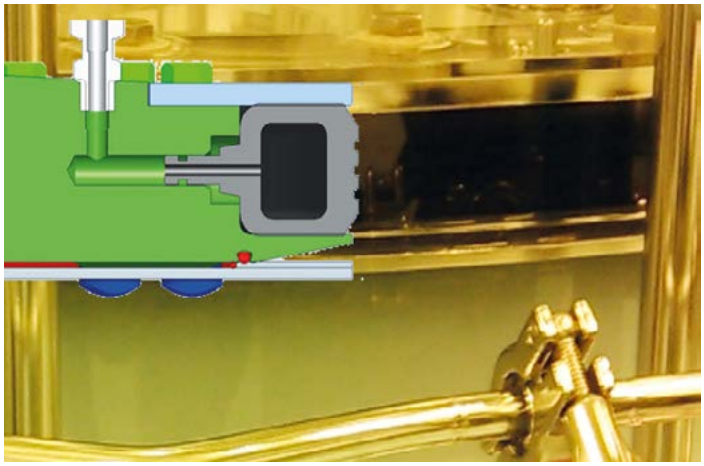

Figure 2: picture and cut-away view of the InPlace top adapter

Column packing

The InPlace™ column of VERDOT Ips^2 being equipped with slurry valves, the media was transferred in "close" conditions minimizing the risk of contamination. The media transfer performed via suction with a controlled stroke allowed transferring a precise amount of media, for ensuring packing reproducibility. The column degassing was instantaneously obtained by actuating the embedded column tilter while deflating the dynamic seal. The media was simply packed in axial compression by lowering the adapter at controlled speed and automatic height monitoring for precise compression control.
This ensured a high performance packing. For instance, an agarose based media bed gave a reduced HETP (Height equivalent to theoretical plate divided by mean particle size) of 2.16, with 4400 plates/m and an asymmetry of 0.96, all within BioMarin acceptance criteria.

Column unpacking

The packed bed was re-slurried in place by injecting one column volume of water alternatively in up flow and down flow, followed with a 20 minutes air-sparging by simply injecting low pressure air through the bottom process connection. For emptying the column, the column was tilted and the slurry valve located at the lowest point of the tilted column was opened to the slurry tank. Air sparging was maintained during the slurry transfer to keep the media suspended, maintain slight pressurization and help the media flow toward the slurry valve. Only small traces of media were left in the column, easily removed with a few liters of water. As result, less than 2.5 columns volume were required to reslurry the bed transfer the slurry back into the tank (maintaining a 50/50 slurry) and completely rinse the column, leaving no trace media.

After validation by VERDOT Ips^2 and BioMarin process experts, BioMarin Operating teams took over and repeated the procedures without assistance to ensure documentation was sufficiently detailed and precise to ensure reproducible results with the different teams working around the clock.

Conclusion

This experience has confirmed that the equipment intrinsic performance is only one success factor of a successful new process implementation. A thorough collaboration between the users and the equipment supplier all along the project, and especially at the project start-up is also paramount for the success.
The very open discussion and confident share of information between the different parties, under non-disclosure agreement, has finally made the collaboration efficient.
As result, it contributed to the timely manufacture of the first lots of rhTPP1 under the early access program and smooth step-up to the forth-coming manufacturing phase.

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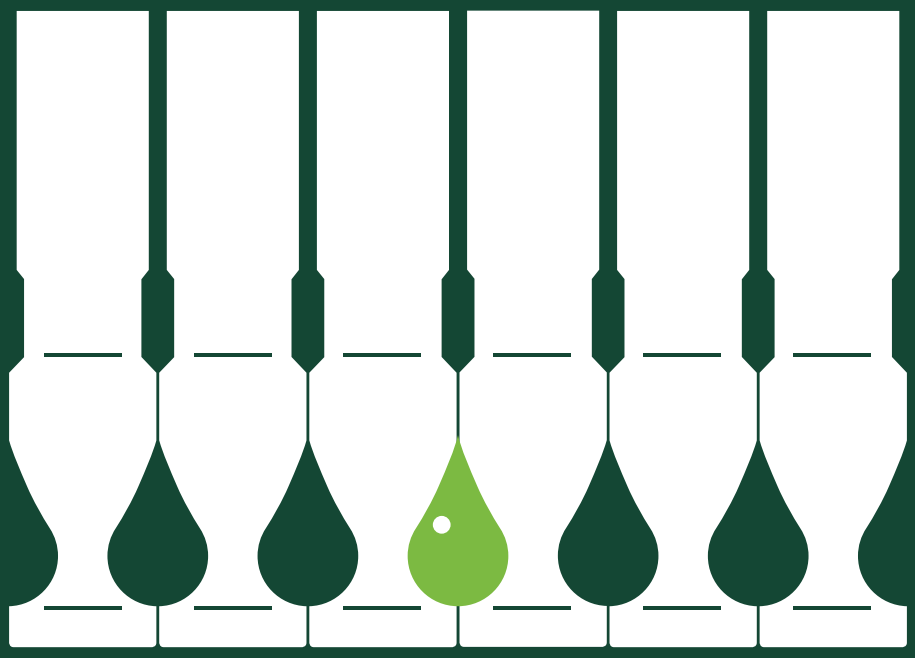

Improving Single Use Bioreactor Design and Process Development.

New Research Towards Intensifying Seed-Train & Scale-up Methods Using 5:1 Turn-Down

By Nephi JONES - Thermo Fisher Scientific
nephi.jones@thermofisher.com

Immense pressure is being applied to improve process knowledge and execution for those working in the field of bio-therapeutic manufacturing. Bioprocess developers are being tasked to provide more product yield at lower production cost, to decrease the time required to bring new therapies to the patient, and to consistently manage operational risks.

These trends are leading to increased focus on three key areas of advancement – continous manufacturing, intensification of the bioprocess unit operations, and increased utilization of single-use technologies.
For animal cell culture processes, the reliance upon a bioreactor as the heart of the upstream operations has necessitated the improvement of these unit operations.

Bioreactor cultures are time and cost-intensive operations, they are also inherently susceptible to contamination or control failures. One method of mitigating the inherent risk of contamination within bioreactor cultures is through the application of Single-Use Bioreactors (SUBs). SUBs have existed in various forms for well over a decade, but it was not until the emergence of Stirred Tank Reactor (STR) with robust film construction, powerful agitation, and reliable in-process analytics did SUBs become a scalable solution. Combining these benefits with significant gains in media and clone productivity has solidified the SUB as a practical alternative for full-scale commercial manufacturing. Significant changes in SUB design have occurred since 2006 when the original 50L and 250L HyClone SUBs were first brought to market; there are multiple manufacturers now offering highly capable SUBs with terminal operating volumes at or exceeding 2000L. ⇢

This trend has reduced the demand for the large stainless steel bioreactor and the burgeoning facility built around expansive water, steam, and cleaning equipment. Recently, the demand for both improved timeline and economics has driven experts in the field of bioprocessing to deviate from traditional scaling approaches. This new trend leverages smaller, more modular facilities and parallelization of 6 X 2000L SUBs in place of single 10-15kL stainless steel bioreactors.

In addition to this change, two approaches can be employed to potentially intensify process focus. The first method incorporates a perfusion device to concentrate productivity into a smaller vessel. Typically an alternating tangential flow filter or standard tangential flow filter are employed on a SUB. One method is to create higher density starting cultures; reducing the time that larger bioreactor is dedicated to producing a single batch. The second method, true perfusion, involves holding the bioreactor near a desired steady state of cell density by managing media exchange, waste removal, and cell bleed volumes in such a way that the cell mass is both well managed in terms of critical process control parameters. The culture is intentionally manipulated to focus on production of the molecule of interest instead of just cell propagation.

Aside from perfusion, the second significant area of focus in bioprocess development is the intensification of the seed train and scale-up operations. Operating bioreactor vessels at low working volumes (high turn-down ratio) is desirable for certain application; however, this mode presents challenges in regard to mixing, mass transfer, and process control. Standard Operating Procedures regarding cell propagation have a common criterion which is often independent of the media type or a particular clone of interest. The requirement is a minimum seed cell density of ~250,000 cells per mL; this often dictates how many reactors vessels are required to reach the "n" or production mode bioreactor. To this end, years of research at Thermo Fisher Scientific have culminated towards optimizing cell culture equipment and methods to improve performance and produce robust process control strategies when operating under these special conditions which we call 5:1 SUB.

Impacts of enhanced energy transfer - Implementing bottom heat exchange, alternate impeller positions, and considering agitation dissipation rates.

In order to achieve consistent results and maintain scalability for cell expansion, it is important to ensure proper mixing within the vessel. This requires that the impeller be well immersed in fluid and the use of the agitator should result in an acceptable mixing time, providing an even distribution of cells and nutrients while minimizing process gradients and localized mixing shear. Selecting an agitator RPM based upon power input to volume (P/V) is the most broadly accepted approach for suspension scale-up and has proven effective for scaling in the SUB. When the vessel volume is at 20% of the rated working volume, it is important to note that the volume of agitation is proportionally much larger; therefore, the average energy dissipation rate is much higher in the 5:1 scenario. Additionally, positioning of the heat exchanger and sensors (RTDs, pH, dO_2) in the bottom of the vessel should be considered mandatory in order to regulate batch temperature and other process values. The weight of the bag will be almost entirely on the vessel floor when the bioprocess container is in the under-filled state, allowing for direct contact with the bottom-mounted heat exchanger.

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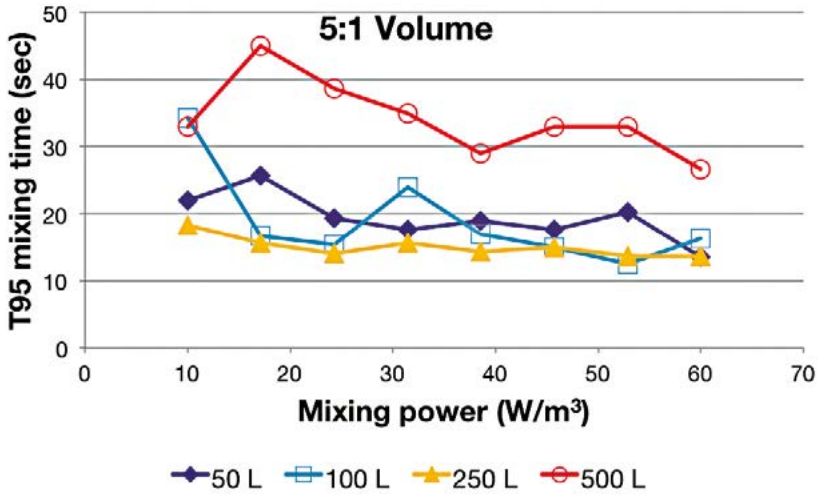
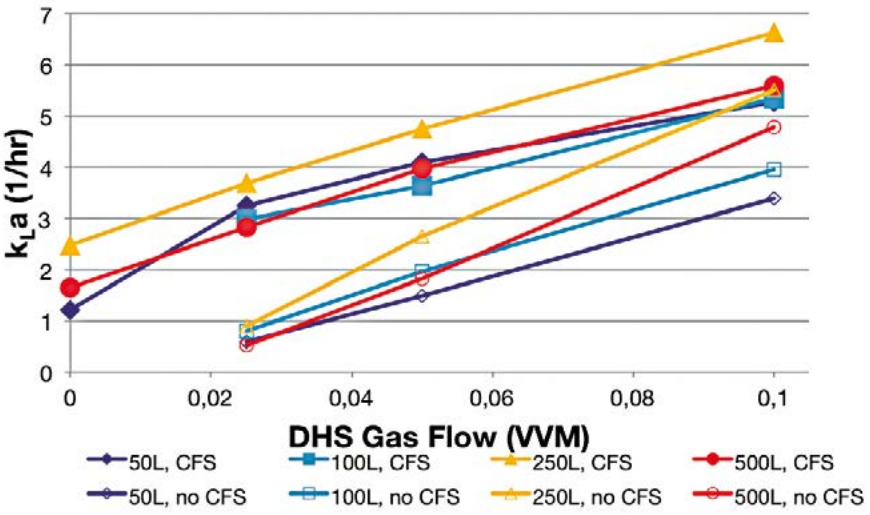


The following data represents t95 mixing times of the 50L 5:1 SUB at 10L, 100L 5:1 SUB at 20L, 250L SUB at 50L, and the 500L 5:1 SUB at 100L.

The following data represent the CO_2 mass transfer performance with and without the CFS at 20% WV in each SUB with corresponding drilled hole sparger gas flow rates.



Maximizing your platform scalability- Taking advantage of the unique Thermo Fisher Scientific Drilled Hole Sparge design and implementing a new Cross Flow Sparge into the headspace have yielded reliable mass transfer and cell culture results.

Traditional STR impeller and sparge designs are derived from legacy systems containing stainless steel fabrication and employed in microbial fermentation. Even glass vessels commonly used at bench scale include spargers constructed from stainless steel. Because the future trend in bioprocessing is directed towards the incorporation of bioreactor parts made from disposable materials, there is interest that critical process components, such as spargers, be preferentially constructed of polymers. There are several parameters that must be examined when modeling and interpreting mass transfer data for cell culture. Thermo Fisher has published our protocols in an effort to drive standardization of k_La characterization. We advocate with our customers in being able to accurately compare variables across different bioreactor designs and configurations, which is critical for improving knowledge and best practices. We have identified the following variables as being significant to system design and influencing characterization results.
A robust protocol should determine the ratio of oxygen to carbon dioxide mass transfer and include the critical parameters.

Methods: probe response time, ambient temperature and pressure compensation, replicates n≥3, randomization, and numerical analysis).
Materials: matching media properties (ions, surfactants, and viscosity), surface energies of spargers (polymers interact differently than metal), kinetics and turbulence at point of bubble entry, bubble size and distribution effects due to impeller speed/position.

A unique feature of the Hyperforma SUB is the use of two highly characterized and best-in-class sparge technologies. First, the Cross Flow Sparge (CFS) is particular to the 5:1 SUB and is a solution to hindered cell culture growth under normal direct sparge and overlay conditions. It was discovered that a lack of process robustness occurred even when pH and dissolved oxygen levels were within specification at their respective points of measurement. The root cause of this reduced cell growth was identified by the presence of a carbon dioxide layer above the liquid. Because this gas is nearly 50% heavier than air, a blanket of CO_2 was generated that could not be eliminated the traditional overlay. The solution to removing this CO_2 layer required the incorporation of a gas line set with sterile gas filter and check valve onto the BPC, positioned within 12 inches above the liquid surface. At start-up, the operator connects the overlay gas supply line to this lower port on the bag (it remains there while at ~20% WV).
Once the culture is ready to expand to 50% working volume, the operator simply moves the gas supply line to the normal overlay location at the top of the SUB-BPC.

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The second sparge technology, the Drilled Hole Sparge (DHS), is unique in that it is the first sparge mechanism that supports STR scale-up via ratio-metric mass transfer characterization. The DHS design was optimized through detailed investigation of bubble theory with analysis of each SUB based upon media type, column height, and agitator interaction. The final design incorporates precision laser fabrication of a film disk with each pore of the sparger having a consistent pore size within a few microns. The pore size and corresponding bubble size (bubble surface area) were matched to the composite residence time or column height of the vessel. The number of holes in each vessel is also scaled to gas entrance velocity (GEV), yielding conservative cell shear levels at 0.1 vessel volumes per minute (VVM). This approach eliminates turbulence and unintended coalesce that is inherent with traditional sparger scale-up.

The resulting performance of the DHS falls between dual sparge configurations (less oxygen k_La than a frit/microsparge and more carbon dioxide k_La "stripping" than an open pipe or traditional 1mm Drilled Hole Spargers). Unlike traditional spargers, the DHS produces sufficient CO_2 stripping at all flow rates. Additionally, it also provides uniquely linear mass transfer performance across all scales of SUB. Of particular note, application studies thus far show that cell culture systems can be operated robustly with pure oxygen as the primary gas without a frit – producing lesser amounts of stable foam at all scales while providing adequate dCO_2 stripping even at the challenging 2000L scale.

Improving bioprocess production – How new technology and methods improves equipment utilization, scheduling efficiency, inventory logistics, and reactor harvest consistency

Intensified processing such as intra-vessel cell expansion via a 5:1 SUB offers huge potential for reducing process risk, reducing scale-up time times, lowering operating costs, and increasing facility throughput. Furthermore, the need for fewer vessels will also reduce inventory of consumables and spare parts. This will drive better standardization and streamline activities related to inventory, quality, and procurement efforts. Energy and risk previously put into preparing extra reactors, making sterile connections, process calibrations, and samplings can all be significantly reduced, allowing operators to invest time towards value added activities. From the process systems standpoint, a robust 5:1 SUB platform will reduce the required floor space for cell culture. In practice, this may double the production capacity of a given suite, which can be a huge paradigm shift in terms of facility utilization. The industry has not likely seen these magnitude of gains since SUBs first began to displace conventional vessels and SIP/CIP skids.

When the cell culture process is ready to be terminated, it is critical that the clarification and harvest step be well executed to not jeopardize the quality of the final product. It is common for large 1-2kL SUBs to consume an 8 hour labor shift during the harvest via centrifuge or DFF systems. The ability to keep the process well mixed down below the 50% working volume helps to reduce plug flow and maximum available separation capacity/investment, and has the potential to reduce product hold-up, improving the overall process yield.

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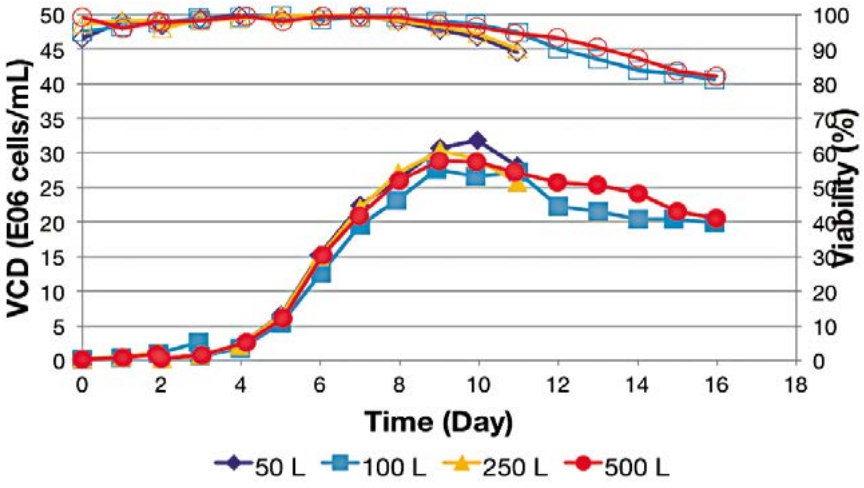

The following data represents a fed-batch culture with CHO-S grown in Dynamis and Efficient Feed C+ Media in the 50L, 100L, 250L, and 500L SUBs. Cultures were initially seeded at 20% WV and scaled to full working volume on day 2 or 3 of culture. Growth rates and productivity levels were maintained across 4 different systems representing a full magnitude of scale-up.

Operating at 5:1 Turn Down with the new 50L, 100L, 250L, and 500L Hyperforma SUB – seed train and pilot scale pre-clinical operating considerations, design features, process characterization, and fed-batch cell culture performance results.

One primary goal of this development was to increase availability of 5:1 technology for end users. Therefore, we wanted to offer a retrofit option to a majority of existing SUB vessels. The existing Hyperforma vessels can be fitted with a conversion kit that includes a longer shaft and modified motor mount block. The new 5:1 SUB BPC has a longer impeller sheath for the agitator. The resulting product offers an increased breadth of operational range and the potential for slightly better k_La due in part to better bubble distribution with a negligible difference in mixing performance. The 50L will likely be the most popular size because it can be seeded at 10L directly from a single incubator flask, eliminating up to 2 seed train vessels. It also provides enough capacity to seed a 2000L SUB 5:1 initiating at 400L.

Operating at 5:1 Turn Down with the new 1000L and 2000L Hyperforma SUB - cGMP operating considerations, design features, process characterization, and fed-batch cell culture performance results.

Our other primary goal was to bring the 5:1 SUB functionality and productivity enhancements into a rigorous cGMP regulated bio-manufacturing environment. Based upon customer feedback and in consideration of the significant SUB installation base already using the legacy SUB platform, we realized the design might need to offer more options. The final concept supports both legacy and 5:1 agitator configurations. This was achieved by capitalizing on the flexibility of the bag whereby a mechanical actuator provides a simple and repeatable method for moving the entire agitator mechanism with the bag (both motor and agitator assembly). This approach provides a more optimal system based upon the following scenario:

1. move agitator down for 5:1 (seeding at 20% WV),
2. move the agitator up (full scale cultivation)
3. later to lower the agitator down at the end of the run (enhance harvest uniformity).

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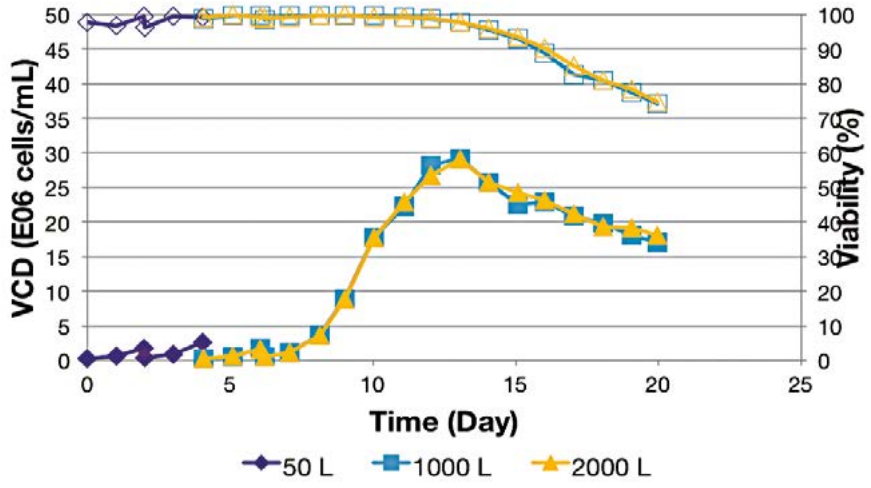

The following data represents the 50L 5:1 SUB starting at 10L, expanding to 50L, and then seeding into parallel 1000L 5:1 and 2000L 5:1 SUBs in this fed-batch CHO-S qualification run.

Conclusion

All the SUBs were originally qualified with conventional batch culture processes. In recent years, we have seen the vast majority of customers transition to include fed-batch and perfusion processes. Moving to a more aggressive process has been challenging for some bioreactors that utilize single impellers. The strength of the Hyperforma design is that it is a direct drive impeller suspended up to 1 impeller diameter off the vessel floor. This provides good mixing distribution at full working volume and the necessary torque capacity for fed-batch or perfusion, yielding sufficient mass transfer when combined with the DHS. At large scale (>500L) having the ability to reposition the agitator or utilize multiple impellers becomes a significant process advantage. The 5:1 design with the single impeller approach allows for simplified feed strategies as the operators do not have to be concerned about partial liquid coverage of the impeller which can lead to excessive splashing or high shear conditions impacting cell culture health. The data presented here demonstrates the scale-up capacity and flexibility of 5:1 SUB platform. End users are clearly excited about the capacity improvements that new technologies are enabling through reduction of manual intervention, complexity, operating costs, and process risk in bioproduction suites.

Qualification approach for the validation of real-word shipping in single-use systems.

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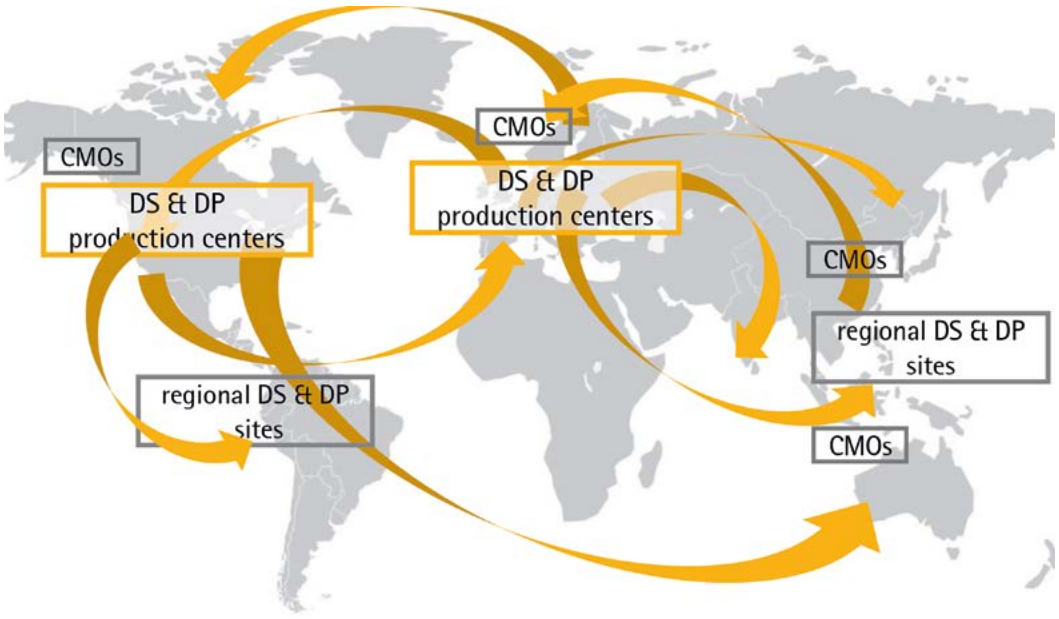

Figure 1. Global manufacturing Network

The complexity of biopharmaceutical manufacturing processes requires continuous improvement. As shown in figure 1, the expansion of manufacturing capacity worldwide has resulted in the multiplication of links between production facilities as well as the increasing need for storage or transportation of media, intermediate, BDS, and drug products between sites over the world.
Outsourcing to contract manufacturing organizations (CMOs) offers a solution to the capacity constraints and reduces the total risks associated with building internal capacity; however, a robust and validated manufacturing process[2], including product transportation between facilities, is then required.

Single-use technology (SUT) continues to expand because of its potential for reducing both capital and operating expenses. The growing adoption of single-use, especially in critical process steps, has increased the need for product quality, robustness, and integrity. Depending on the manufacturing process organization and the level of outsourcing, the challenge of safe and robust BDS transportation becomes a crucial step from a risk analysis point of view. These shipments, increasingly performed in single-use bags, require thorough validation protocols to demonstrate their integrity and protection of the drug substances. The ASTM[3] and ISTA [4] both provide standardized validation methods using a variety of simulated shipping hazards. These tests are qualitative by nature with simple pass-fail criteria, therefore it is important that the robustness and failure modes of shipping systems are evaluated under real transfer conditions.

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Regulatory background

Regulatory agencies like FDA[5], EMA[6] or EU[7] emphasize the need for end-user to ensure that their drug processes produce consistent and reproducible results which meet the quality standard of the drug product. Validation is "Establishing documented evidence that provides a high degree of assurance that a specific process" including shipping "will consistently produce a product meeting its pre-determined specifications and quality attributes" (FDA).
A properly designed system will provide a high degree of assurance that every process step, including shipping has been properly evaluated before its implementation.
In the biopharmaceutical industry, qualification and validation are intended to demonstrate that the manufacturing process provides the desired level of compliance of the product and specifically its activity, sterility and potency. Qualification of a shipping system and equipment is part of the process validation.
According to the PDA technical report N°66[8], "Shipping systems must be qualified for their intended use through proper design and testing in consultation with a packaging engineer. The transportation routes must be defined for international shipment. A risk assessment for vibration, handling, delays and seasonal variation should be established".

International standards description

ASTM or ISTA are well-known standards for shipping systems. They are aimed to compare or evaluate the effectiveness of protective packaging and / or a packaged-product's ability to withstand the hazards of distribution. Table 1 briefly describes the main features of ASTM and ISTA standards.
The level of severity must be defined according to real shipment condition in addition of desired safety margin. There is no official ASTM claim and suppliers can only claim that they pass ASTM selected tests described by the standard. Therefore, knowing the distribution cycle, schedule, duration, severity level, and acceptance criteria are mandatory to understand the validation performed on the system.
Moreover, the suitability with the intended use can only be proven by end-users; these conditions might differ from one site to another or from one product to another.

The definition of the system to be tested and the testing program need then to be carefully assessed and justified. The selection of the right testing parameters and the tested samples' configurations can be done only by knowing the transportation cycles and the type of impact perceived by ⇢

ASTM D4169 – Standard Practice for Performance Testing of Shipping Containers and Systems	ISTA 3-Series: General Simulation Performance Tests
General Simulation tests covering a range of package types and distribution scenarios. The user must choose from tests, alternatives, intensities, sequences and specific procedures based on packaged-product and distribution characteristics. Applicable across broad sets of circumstances, such as a variety of vehicle types and routes, airplane, boat, rail, or a varying number of handling exposures. Tests are carried out sequentially on the same package. 18 Distribution Cycles (DC): DC should be chosen close to the projected distribution, like for example: – Preconditioning and conditioning – Handling – Shock (Horizontal impact, Rotational flat drop and Edge drop) – Vibration truck – Low pressure – Air vibration – Compression (optional) Three levels of severity (I, II, III) are described in the ASTM D 4169	Designed to provide a laboratory simulation of the damage-producing motions, forces, conditions, and sequences of transport environments. Applicable across broad sets of circumstances, such as a variety of vehicle types and routes, or a varying number of handling exposures. For example: 3E & 3H tests consist of 7 to 15 individual tests that are carried out sequentially on the same package. The test simulates the handling and transit required in a road distribution network and covers truck transport only. It is composed of sequences including for example: – Preconditioning and conditioning – Shock (Horizontal impact, Rotational flat drop and Edge drop) – Vibration only truck – Compression (optional)

Table 1. ASTM and ISTA standards

the load during the transportation, thus requiring preliminary testing and analysis.

Qualification approach

The supply of process solutions in large-volume bags, from point of manufacture to point of use is a well-established practice that involves the following elements:

- A bag designed to fit a rigid wall outer container
- A rigid wall outer container such as a plastic drum or tote or a stainless-steel bin
- Secondary packaging materials (e.g., dunnage) and lids or other mechanical devices to suppress the fluid wave action in the bioprocess bag.

Transportation of process solution in small-volume bags is also a common process that requires less complex packaging solution. The exception is the transportation of frozen materials that necessitates temperature-resistant materials and cold-chain logistics.

The PDA TR 66 has highlighted specific factors of importance for transportation that must be considered by end-user. These factors are:

- Dimensional factor (i.e., volume to be shipped and dimensions of the shipper)
- Mode of transportation, whether it's ground, air, rail, boat, or combination of more than one mode of transportation. Metrics must include hold time on tarmac
- The associated environmental conditions (temperature, humidity, pressure, and variation)
- Functionality (i.e., forklift access, stack ability of outer container, access to fill and drain port, secondary container to collect leak)
- Room classification such as fill and drain procedure to maintain sterility
- Logistics (e.g., external shipper, cold-chain logistics).

SUT shipping system composed of a bag and a stainless-steel bin should ensure safe shipment (i.e., no loss of integrity and no loss of product sterility). It can be granted by the mechanical robustness of the shipper. The objective is to verify that no leaks occur during transportation.

Qualification and validation of the shipped product can be performed in real shipment with monitoring or in simulated shipment according to ASTM D4169 or ISTA. ⇢

Figure 2. Typical transportation sequence between plant A and plant B

The protocol of test needs to correlate to the projected life cycle phase of the shipped unit. As a first step, knowledge of shipped product and the type of transportation (mean and sequences) are key to understand the shipping cycle and provide the adequate safety margin during qualification testing program.
A typical distribution sequence between two plants in the biotech industry is described in figure 2.

The choice of the testing program needs to fit the type of tested load and the parameters needs to be determined with a rational based on the shipping conditions and/or experience and packaging expertise in order to understand the impacts by transportation phases.

The next step is to define the severity of testing (level and duration). The level of severity should been defined according to a desired safety margin chosen over the real shipping tests impacts. The combination between real shipping tests and laboratory tests for the system characterization with ASTM D4169 and ISTA3E provides the relevant data helping to select the appropriate qualification method, parameters and acceptance level to qualify the shipping system (bags + shipper).

Conclusion

Shipping is indeed complex and the user should not be assuaged simply by vendors' claims about regulations (i.e., claims of being "ISTA certified" or "ASTM compliant"). It is important to also understand what is behind each claim and verify that it is applicable to the product's intended use. The end-user should understand the trial conditions used in the vendor tests and compare them to its application. The acceptance criteria (bag and shipper), the protocol, and trial conditions shall be discussed.
Shipping validation needs to be carefully defined in close collaboration between end-user and vendor, with parameter setting linked to actual use. Collecting vibration data on the real use will help the end user and the vendor to understand the physical constraints of the shipping mode and select the best protocol to replicate them in laboratory testing. The limits of the system should be defined with knowledge of the safety margin and be tested under real packaging and real transport conditions.

References

[1] N. Riesen, R. Eibl, "Single Use Bag Systems for Storage, Transportation, Freezing, and Thawing" in Single-Use Technology in Biopharmaceutical Manufacture, Dieter Eibl and Regine Eibl Eds. (John Willey & Son, Hoboken, NJ, 2011.
[2:] S.D. Jones and H. Levine 2005, BioExecutive International 3, 2–5, 2005.
[3:] ASTM (American Society for Testing Materials) D4169: Standard Practice for Performance testing of Shipping Containers and Systems, 2014
[4] ISTA (International Safe Transit Association): General Simulation Performance tests
[5] FDA, Guidance for Industry Process Validation: General Principles and Practices, January, 2011.
[6] EMA,Guideline on Process Validation for the Manufacture of Biotechnology derived Active Substances and Data to be provided in the Regulatory Submission, April 2014.
[7] EU, European Commision, EU guidelines for Good Manufactruing Practice (Brussels, February 2014).
[8] PDA, Technical Report N°66, "Application of Single-Use Systems to Pharmaceutical Manufacturing", 2014.

Expansion of Human Bone Marrow-Derived Mesenchymal Stem Cells in BioBLU 0.3c Single-Use Bioreactors.

By Vincent DUFEY, Aurélie TACHENY, Muriel ART, Ulrike BECKEN & Françoise DE LONGUEVILLE - Eppendorf
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Mesenchymal stem cells (MSCs) are attractive candidates for therapeutic applications, especially in the field of regenerative medicine [1] because – in contrast to embryonic stem cells – they do not pose ethical issues, they can be isolated from various tissue sources, and they reduce the risk of rejection reactions. The doses of human MSCs (hMSCs) needed for clinical trials are estimated at between one and 200 million cells per patient, depending on the disease being tackled [2]. One of the most important challenges in providing hMSCs for curative use is the production of large quantities of cells in a robust manner.

Indeed, whatever the tissue source, the number of hMSCs extracted is very low, and not sufficient for clinical use; hence the hMSCs have to be expanded following isolation. Besides providing the needed cell quantities, hMSC production must also comply with the manufacturing process regulations required of a fully controlled production system. hMSC expansion in stirred-tank bioreactors can be monitored and is scalable, and hence can fulfill these requirements from experimental quantities to production. In the study presented here, we used an Eppendorf DASbox Mini Bioreactor System equipped with BioBLU 0.3 Single-Use Vessels to culture adherent multipotent stem cells on microcarriers, and reached a clinically relevant number of cells.

···→

Material and Methods

Culture of human bone marrow-derived mesenchymal stem cells (hMSCs-BM)

We cultured hMSCs-BM (Lonza) at 37°C in MSCGM Mesenchymal Stem Cell Growth BulletKit Medium (Lonza), including both the basal medium and the necessary supplements. The cells were first expanded in Eppendorf Cell Culture Flasks. At passage six, we harvested the cells to be used for three-dimensional microcarrier culture in a stirred-tank bioreactor.

We processed the experiments in parallel in a 4-fold Eppendorf DASbox Mini Bioreactor System for cell culture, equipped with Eppendorf BioBLU 0.3c Single-Use Vessels. We inoculated the cultures with hMSCs-BM combined with Cytodex type 1 (GE Healthcare Bio-Sciences) or Cytodex type 3 (Sigma-Aldrich) microcarriers. The initial number of cells per bioreactor was 6 x 10^6 cells, which corresponds to a cell density of 9,700 cells/cm^2 for Cytodex type 1 and 11,000 cells/cm^2 for Cytodex type 3.

The initial working volume was 100 mL. To promote the initial cell adhesion, we did not agitate the culture for 24 hours. After this attachment period, we manually adjusted the culture volume to 200 mL, and set the agitation speed to 60 rpm for the entire proliferation phase. The pH of the growth medium was controlled at 7.6 by automatic addition of CO_2 in the vessel headspace. The dissolved oxygen (DO) level was maintained at 40 % by delivering gas (N_2, air, and O_2) into the medium with the gas flow set at 0.1 sL/h. To maintain the culture until its maximum yield, we exchanged 50 % of the culture medium after 6 and 8 days of culture. After 9 days, up to 70 % of the medium was replaced almost daily. During those refreshment steps, agitation was stopped to let the microcarriers sediment.

The cells were cultivated for 20 days on Cytodex type 1 and for 27 days on Cytodex type 3 microcarriers.

Analysis of cell viability, cell counting, and metabolite measurement

During the proliferation phase, we regularly analyzed the cell viability on the microcarriers by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) coloration.

To evaluate cell growth, we collected samples of the cell/microcarrier suspensions, detached the cells from microcarriers and determined the cell number using the CASY Cell Counter and Analyzer, model TT (Omni Life Science). We used the supernatants collected during cell counting to quantify glucose and lactate concentrations, using the Glucose RTU Kit (Biomérieux) and L-Lactate Assay Kit (Abcam), respectively.

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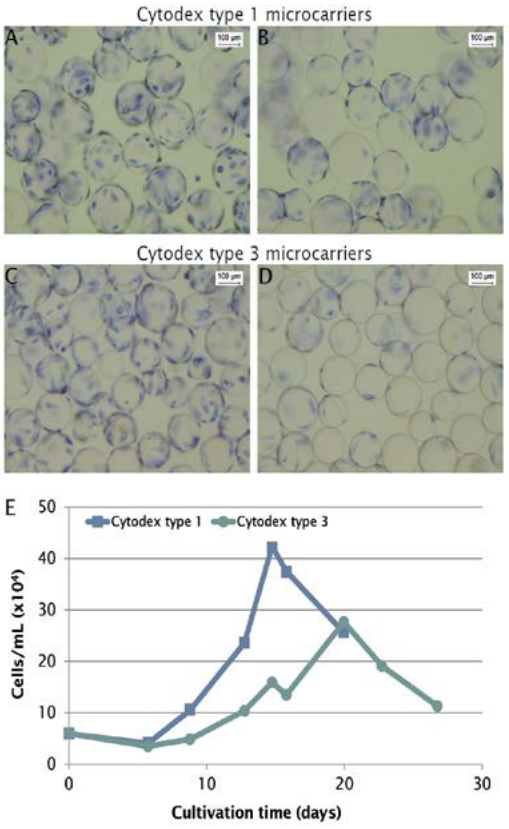

Fig 1: hMSC-BM growth on microcarriers.

A-D: MTT-coloration of hMSCs-BM. Upper panel: Cells were cultured on Cytodex type 1 microcarriers and stained before (A) and one day after (B) the addition of fresh beads. Lower panel: Cells were cultivated on Cytodex type 3 microcarriers and stained before (C) and one day after (D) the addition of fresh beads.

E: hMSC-BM growth profiles in BioBLU 0.3c Single-Use Vessel with Cytodex type 1 and Cytodex type 3 microcarriers. Mean values of two experiments carried out in parallel are shown.

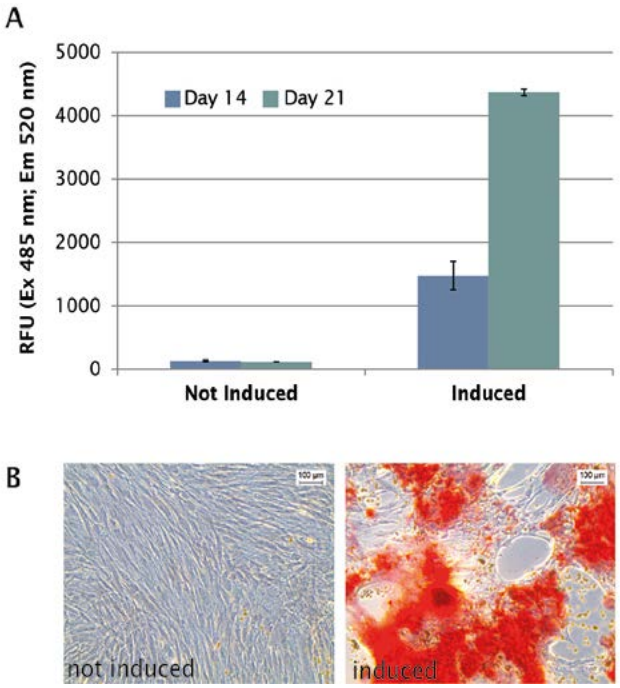

Figure 2. Osteogenic lineage differentiation

A: OsteoImage Mineralization assay with hMSCs-BM cultured on Cytodex type 1 microcarriers after 14 and 21 days of osteogenic lineage induction. Means and standard deviations are shown (n=3 replicates). RFU: Relative fluorescence units. Ex: Excitation. Em: Emission.

B: Alizarin red S staining of hMSCs-BM cultured on Cytodex type 1 microcarriers. Negative control (left) and induced cells (right) on type 1 microcarriers. A representative result is shown.

hMSC-BM differentiation

We detached hMSCs-BM from microcarriers by trypsin treatment, and seeded them into BD BioCoat Fibronectin Cellware, 24-well plates (Corning). We induced differentiation of the hMSCs into mature osteoblasts and chondrocytes, respectively by replacing the expansion medium with hMSC Osteogenic Differentiation BulletKit Medium and Chondrogenic Differentiation BulletKit Medium (Lonza), respectively.
At days 14 and 21 we assessed the bone cell mineralization using the OsteoImage Mineralization Assay (Lonza). Moreover, at day 21 we stained calcium deposits with an anthraquinone dye (Alizarin Red S Staining Kit, ScienCell). At day 14, we detected proteoglycans secreted by chondrocytes by Alcian Blue staining (Sigma-Aldrich).

Results

hMSC-BM expansion on microcarriers in BioBLU 0.3c Single-Use Vessels

To investigate the suitability of BioBLU 0.3c Single-Use Vessels for the scalable production of multipotent stem cells, we cultured hMSCs-BM in parallel on two microcarriers types. Cytodex type 1 microcarriers are based on a dextran matrix covered with positively charged groups, while a layer of denatured collagen is covalently bound on the Cytodex type 3 dextran surface. One possibility to expand cells in a microcarrier culture is the use of a technique called bead-to-bead transfer. By adding fresh microcarriers into the existing culture, cells can switch from one carrier to another, and start to grow on the empty beads[2, 3, 4].
This allowed us to avoid subculturing techniques traditionally used with adherent cells. When stained cells were visible on all beads (detected by MTT coloration) we added fresh microcarriers to offer additional growth surface (Fig. 1A-D). We obtained the best proliferation rate on Cytodex type 1 microcarriers (Fig. 1E). The cell number increased 17.5 fold to a maximum cell density of 1 x 10^8 cells/bioreactor at day 14, corresponding to 4 x 10^5 cells/mL. On Cytodex type 3 microcarriers, 20 days of culture were needed to reach a maximum cell number of 7 x 10^7 cells per bioreactor, which is 11.5-fold higher than the initial seeded quantity and corresponds to 2.5 x 10^5 cells/mL. Glucose monitoring revealed a rapid nutrient consumption of the hMSC-BM culture on both microcarrier types.
As a result of glucose consumption and feeding, the glucose concentration varied in a characteristic pattern, but never dropped below 0.1 g/L (not shown). As by-products of their glucose metabolism, the cells produce metabolites, such as lactate and ammonia, which accumulate in the medium (not shown). High lactate concentrations can affect hMSCs growth as well as cell morphology. We maintained the lactate concentration below 25 nM to ensure that a concentration of 35.4 nM, which was found to inhibit growth[5], was never reached.

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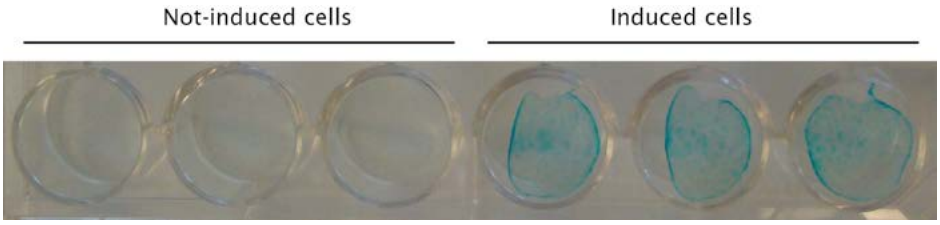

Figure 3. Chondrogenic lineage differentiation. Alcian Blue staining of hMSCs-BM cultured on Cytodex type 1 microcarriers after 14 days of chondrogenic lineage induction.

Multipotency analysis of hMSCs-BM

To confirm that hMSCs-BM cultured in BioBLU 0.3c Single-Use Vessels retained their differentiation capacity, we used them for in vitro osteogenic and chondrogenic differentiation assays. We obtained equivalent results with cells cultured on Cytodex type 1 and 3 microcarriers. Osteogenic differentiation is divided into three stages: cell proliferation (from day 1 to 4), extracellular matrix maturation (from day 5 to 14), and finally, matrix mineralization (from day 14 to 28). This last step is characterized by high expression of osteocalcin and osteopontin, followed by calcium and phosphate deposition[6]. Bone is composed of the organic protein collagen and the inorganic mineral hydroxyapatite. Bone mineralization can be quantified by specific staining of the hydroxyapatite portion. Bone mineralization was detectable already after 14 days of induction and was clearly increased at day 21. Hydroxyapatite was only detected on induced cells (Fig. 2A). After 21 days of osteogenic lineage induction, calcium deposits, indicating the matrix mineralization phase, were furthermore detected by Alizarin Red S staining on induced hMSCs-BM, while we observed no deposition in the not- induced negative control (Fig. 2B). Next we assessed chondrogenic differentiation, which is a complex, multi-stage process characterized by the production of cartilage-specific molecules such as type II collagen and proteoglycans. Detectable by Alcian Blue staining, proteoglycans are considered to be a marker of cell chrondrogenesis. After 14 days of chondrogenic lineage induction, we detected strong cartilage proteoglycan synthesis, while the level stayed low in the negative control (Fig. 3).

Conclusion

In summary, our results demonstrate that the DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessels is suitable to expand multipotent stem cells in a safe and controlled manner.

Glossary

Ex: Excitation
Em: Emission
hMSC: human mesenchymal stem cell
hMSC-BM: bone marrow-derived human mesenchymal stem cell
MSC: mesenchymal stem cell
MTT: 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
RFU: Relative fluorescence units

References

[1] Wang S, Qu X, Chunhua Zhao R. Clinical applications of mesenchymal stem cells. Journal of Hematology and Oncology Review 2012; 5:1-9.
[2] Jung S, Panchalingam K M, Wuerth R D, Rosenberg L, Behie L A. Large-scale production of human mesenchymal stem cells for clinical applications. Biotechnology and Applied Biochemistry 2012; 59:106-120.
[3] Wang Y, Ouyang F. Bead-to-bead transfer of Vero cells in microcarrier culture. Cytotechnology 1999; 31:221-224.
[4] Sart S, Schneider Y-J, Agathos S N. Ear mesenchymal stem cells: An efficient adult multipotent cell population fit for rapid and scalable expansion. Journal of Biotechnology 2009; 139:291-299.[7] Schop D, Janssen F W, van Rijn L D S, Fernandes H, Bloen R M, de Bruijn J D ven Dijkhuizen-Radersma R. Growth, Metabolism and Growth Inhibitors of Mesenchymal Stem Cells. Tissue Engineering 2009; 15:1877-1885.
[5] Schop D, Janssen F W, van Rijn L D S, Fernandes H, Bloen R M, de Bruijn J D ven Dijkhuizen-Radersma R. Growth, Metabolism and Growth Inhibitors of Mesenchymal Stem Cells. Tissue Engineering 2009; 15:1877-1885.
[6] Huang Z, Nelson ER, Smith RL, Goodman SB. The sequential expression profiles of growth factors from osteoprogenitors to osteoblasts in vitro. Tissue Engineering 2007; 13: 2311-2320.

Single Use & Stainless Steel: complementarity or fight?

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There has been a huge increase in single use apparatus in the biopharmaceutical manufacturing world during the last. Many companies compete in manufacturing production tools. At Boccard where our mantra is "In Stainless Steel We Trust", we have a different opinion. We believe that plastic and stainless steel are complementary. Let's take the example of cell culture. Before growing in a huge fermenter (e.g. 1000 L), what is the point of using a stainless steel 20 L fermenter as a first step from an economical point of view? why not use Single Use apparatus in this case?

As an industrial integrator who has been dealing with Stainless Steel for almost 100 years worldwide, we have been regularly asked on Single Use and Stainless Steel. We notice that when talking about this topic, you easily face pros and cons. Whoever you listen to, the arguments seem legitimate.

Single Use Technology started being available about twenty years ago. Viewed with caution at its start, it rapidly became attractive for many reasons. Firstly Capital Expenditure CAPEX is definitely reduced. Secondly, the availability of the units is higher as no time is needed for cleaning and sterilizing. This eliminates also the use of chemicals and water and diminishes the use of energy as well as environmental footprint.

Cross-contamination is a major concern in biotech. Single Use – as its name suggests– reduces this kind of risk. Qualification and validation procedures of the processes are much easier.

⇢

Table 1: Why is Single Use Technology attractive compared to Stainless Steel?
Minimized CAPEX
No CIP / No SIP
Limitation of the risk of cross-contamination
High Plant flexibility
Reduced environmental footprint
Easy qualification & validation procedures of installation
Reduced maintenance costs

Table 2: Why Stainless Steel remains attractive compared to Single Use?
Fully under control
Lower life-cycle cost
Wide range of sizes
Not Extractable / Not leachable
Autonomous
No plastic required
More comfortable for the operators
Not limited to one technology only

Single Use bioprocessing enables time saving as it can be qualified in advance.
Maintenance is made easier and much cheaper. Last but not least, the plant flexibility is increased and the lead time necessary to get the devices is relatively short. These are additional benefits as time to market is predominant. It is obvious that costs can escalate if the product is not available on time.
Single Use Technology could be seen as showing definitely many advantages compared to the Stainless Steel bioreactors. However, everything is not all black and white.

Arguments in favor of conventional Stainless Steel are numerous too.

We are lucky to be in contact with major players worldwide. Despite the numerous improvements which have occurred in Single-Use Technology, the traditional brick and mortar continues to show a lot of advantages.

First of all, it is clear for everyone that it is well established and fully under control. Secondly, **despite Single Use Technology, there is no capacity limitation**. We design and build tailor-made skids as a response to our clients' specific needs. This is especially interesting when they are manufacturing on a large scale. Maximum size for a single use fermenter bag is 2,000 L.
Do you know many companies willing to take the risk of handling this size or even bigger bags from a practical point of view?
A wide range of capacity is not the only advantage. If we consider life-cycle costs on the whole, it is cheaper to use Stainless Steel solutions. The use of plastic is zero as no plastic bag is required to proceed. In other words and on a long term basis, impact on the environment is smaller. Keep in mind that all the plastics must be incinirated after use.

We previously talked about cross-contamination concern. It is clear that there is no acceptable consensus about the Single-Use extractable and leachable levels. With Stainless Steel, it is simply easier. Of course there is no such thing as zero risk. But once your unit is cleaned and sterilized, risks are undeniably very limited. Moreover operators have less manual activities as they do not have to prepare any manifolds. So the risk of misuse is limited.

There are many more examples which show that Stainless Steel still has its place. Customising the process environment is one of them. A last one is that equipment available on the plant is not limited to one technology only. Indeed, other technologies can be implemented around the existing processes.

We could also develop the arguments against each solution with regard to CAPEX, Operating Expenditure (OPEX), safety, environmental footprint, ergonomics, etc. Whether you are an investor, a producer, an operator, a quality manager, a supplier, or anyone else, the conclusion remains the same: there is room for both technologies.

Whichever company (emerging or very well established), their target remains the same: limited investments and quick productions. For now, it is wise to turn to the Single Use Technology when small tank volumes are required and by mixing the different techs. For a downstream process, buffer preparation will be made with SU bags linked to filtration and/or chromatography skids. Upstream, 20 L SU fermenter is going to a 200 L and to a 1,000L fermenter (both made in Stainless Steel). During the last couple of years we have also observed new SU skids entering this market like filtration or continuous chromatography. These are big changes for our industry, where the most agile only will be able to survive.
And what about the 3D printing: when will the first skid be available?
We believe that in the end, complementarity will be the key for a majority of projects. The agile Industrial Integrators able to integrate all techs, match production scales and address economic issues are likely to remain. At Boccard, we strive to be one of them and still... In Stainless Steel We Trust!

Glossary
SU: Single Use
CIP / SIP: Clean In Place / Sterilization In Place
CAPEX: Capital Expenditure
OPEX: Operational Expenditure

Bibliography

- The Medecine Maker x GE Healthcare: The Great Debate Stainless Steel Versus Single Use, September 2016
- BioProcess International: The Single-Use or Stainless Steel Decision Process: A CDMO Perspective, December 2015

2017, des Sessions toute l'année !

Dans les 5 domaines spécifiques au "Propre et Stérile", A3P Formation vous propose des Sessions toute l'année, avec des formateurs/experts reconnus dans chacun de leur domaine !

Maîtrise de la contamination

Bonnes Pratiques de Fabrication (BPF)

Qualification

Process

Systèmes informatisés

Calendrier des SESSIONS*

AVRIL 2017				
	MC02	*Validation des procédés de nettoyage des équipements de production en industrie pharmaceutique*	4 & 5 avril	Pierre DEVAUX
	PROC02	*Lyophilisation 2 : développement et perfectionnement des connaissances*	26 & 27 avril	Dominique SIERAKOWSKI
MAI 2017				
	SI03	*Validation des systèmes informatisés efficace et efficiente*	16 mai	Jean-Louis JOUVE
	MC11	*Stérilisation par la chaleur : principes, validation et production*	17 & 18 mai	Dominique SIERAKOWSKI
JUIN 2017				
	BPF06	*Processus d'agrément et de suivi des fabricants d'excipients Directive du 19 mars 2015 (2015/C95/02)*	1 juin	Olivier JEAN
	SI05	*Audit et résolution des problèmes de "data integrity" au laboratoire de contrôle qualité*	8 juin	Jean-Louis JOUVE
	BPF07	*Rupture de Stock. Comment préparer un plan de prévention*	13 juin	T. ZIMMER & JF DULIERE
	PROC03	*Lyophilisation 3 : expertise et maîtrise des procédés et de la qualité*	14 & 15 juin	Dominique SIERAKOWSKI
	MC08	*Maîtrise de la contamination en ZAC (stérile et non stérile)*	27 & 28 juin	Pierre DEVAUX
	SI04	*Évaluation des fournisseurs IT/IS : outils et pratiques*	29 juin	Jean-Louis JOUVE
SEPTEMBRE / OCTOBRE / NOVEMBRE 2017				
	SI01	*Cloud Computing et réglementation pharmaceutique*	7 sept.	Jean-Louis JOUVE
	MC12	*Autoclave et stérilisation à chaleur humide*	26 sept.	Walid EL AZAB
	BPF03	*Gestion du risque qualité (ICH Q9) des procédés aseptiques*	4 & 5 oct.	Dominique SIERAKOWSKI
	MC07	*Elaboration d'un programme de bio-nettoyage en salles propres en environnement BPF*	12 oct.	Pierre DEVAUX
	BPF02	*Procédés aseptiques : simulation et filtration stérilisante*	24 & 25 oct.	Dominique SIERAKOWSKI
	QUAL01	*Qualification d'une boucle d'eau purifiée*	26 oct.	E. PETAT & S. RINGA
	PROC09	*Maîtrise de la chaîne du froid*	28 & 29 nov.	Agnès FELIX-PICAUT

*sous réserve de modifications

D'autres sessions sont prévues dans l'année.
Merci de nous contacter pour plus d'informations.

Low Endotoxin Recovery (LER) is today one of authorities serious concerns regarding pyrogen testing.

By Dr. Anja FRITSCH - Confarma France SAS

Low Endotoxin Recovery (LER), initially described by Chen and Vinther in 2013, is a phenomenon occurring in protein formulations that is characterized by efficient masking of Lipopolysaccharides (LPS/ endotoxin). Depending on the protein - buffer characteristics, the temperature - and time - dependent masking effect can lead to complete non-detectability of LPS, which might present a serious risk for the patient. At first being interpreted as a purely artefactual event occurring in testing laboratories, LER is today one of FDAs serious concerns regarding pyrogen testing.

Pyrogens – Definition and methods for their detection

Pyrogens: "A substance that induces a febrile reaction in a patient."[1]

Endotoxins: "A pyrogenic product (e.g., lipopolysaccharide) present in the bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections ranging from fever to death. "[1]

Lipopolysaccharides / endotoxins are a subtype of pyrogens originating from the cell wall of gram-negative bacteria that are both extremely thermostable and very potent when brought into contact with the human immune system. But they are by no means the only possible pyrogenic contamination – and the growing complexity of biotechnological products increased the risk of having pyrogens present that originate from a variety of microorganisms and other sources (see table 1). ⇢

Substance
Endotoxin associated proteins
Peptidoglycans (components of bacterial cell wall)
Muramylpeptides
(MDP and other subunits of petidoglycan synergise with endotoxins)
Porins (proteins from the bacterial cell wall)
Bacterial outer surface proteins
DNA (bacterial)
Lipoteichoic acids and further Gram-positive bacterial cell-wall components
Superantigens
Exotoxins
Lipoarabinomannans (from mycobacteria)
Fungal components (for example, mannans, glucans, mannoproteins)
Parasite components (for example, phosphoinositol)
Viruses
Non-microbiological contaminations (for example, cytokines, media, cells, breakdown products)
Solid materials (for example, medical devices, plastic)
Drugs (for example, steroids, bile salts, dapsone, cytokines)

Table 1 : Origins of Pyrogens

History of pyrogen detection

The first mentions of pyrogenic substances date from the 1870s, but it was only in 1914 that pyrogen tests on rabbits were introduced in the British Pharmacopoeia. These tests are based on measuring the increase in body temperature of rabbits after injection of the product being tested. Despite being a rather laborious in vivo assay and being specific for substances pyrogenic for the rabbit, the test was long the reference assay for detection of pyrogenic contaminations.

The first alternative developed in 1970 as a replacement of the pyrogen testing on rabbits was the test for bacterial endotoxin based on extracts of the blood of the horseshoe crab. This test was fast and reliable, but limited to the detection of endotoxin.
In order to have a full replacement of the pyrogen test on rabbits, a new test was introduced in 2000 that was based on the human immune system and its reaction to pyrogenic substances. The Monocyte activation test is able to detect all substances pyrogenic to humans and is expected to replace the rabbit pyrogen test in Europe from 2017.

Up to now, the authorities have accepted the endotoxin test as test for pyrogenic contaminations, but the growing complexity of the new products entering the market has caused a change in the harmonized European and American Pharmacopoeia chapter on pyrogen detection. Acknowledging the growing risk of non-endotoxin pyrogens being present, a new prerequisite for the use of the endotoxin test is now a proof that endotoxins are the only possible contamination of a product or production process. Additionally, the phenomenon of Low Endotoxin Recovery needs to be investigated and the authorities call for strategies to overcome masking effects, whenever they are detected.

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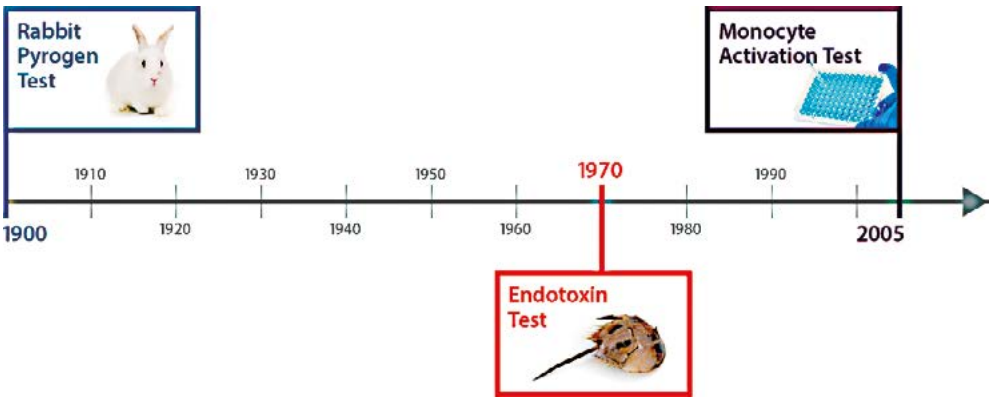


Figure 1: Development of endotoxin and pyrogen detection tests

LER investigation – Hold-time studies

Investigations performed analyzing common protein formulation matrices have demonstrated that buffers containing citrate or phosphate in combination with surfactants like polysorbates are likely to cause LER. Although the mechanism is currently unclear, it has been suggested that the masking effect is due to a change in the structure of the LPS molecule, leading to a decreased binding to the pro-enzyme form of factor C that induces the enzymatic cascade used for detection of endotoxin in the Limulus amoebocyte lysate test (Reich et al.,). One of the many uncertainties today regarding the LER is the extend to which this masking also occurs in the human body, where recognition of the LPS depends on its binding to specific receptors on the surface of monocytes that in turn trigger the febrile response.

Although certain risk factors for the occurrence of an endotoxin masking have been identified, the only means of excluding a LER is currently to perform hold-time studies with endotoxin spiked directly into the sample[3]. The samples are then incubated at different temperatures, usually at 5 ± 3°C and 20 ± 5°C for up to 7 days. If a LER is present, the endotoxin shows a time and temperature-dependent decrease in concentrations, whereby the kinetic of the endotoxin masking depends strongly on the product, its formulation buffer and the source of endotoxin used. At Solvias, endotoxin hold-time studies are performed using the USP endotoxin standard, which corresponds to the expectations of the authorities, although testing with naturally occurring endotoxins may be more representative for the actual masking of real-life-contaminations.

Should a masking effect be observed, we suggest to run hold-time studies using the Monocyte activation test, as in some cases, the masking effect is specific for the endotoxin test and a change of test method is already enough to allow testing for pyrogenic contaminations. Endotoxin demasking agents are available, but development of demasking strategies is currently still difficult, as the mechanism underlying the masking is not quite clear. Changing the sample composition during demasking may also interfere with detection of non-endotoxin pyrogens, if the risk analysis has shown that such may be present and a Monocyte activation test or a pyrogen test on rabbits have to be performed.

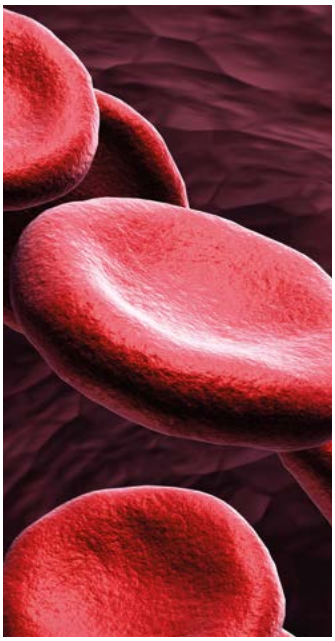
References

[1] Guidance for Industry, Sterile Drug Products, Produced by Aseptic Processing — Current Good Manufacturing Practice FDA 2004
[2] ECVAM Workshop 43: novel pyrogen tests
[3] "Endotoxin recovery using limulus amebocyte Lysate (LAL) assay".

ACTUALITÉS

Mercredi 4 octobre, Lyon

Journées A3P Endotoxines



- LER (Low Endotoxin Recovery)
- Dispositifs médicaux et les endotoxines
- Nettoyage en place et assurance de stérilité

Conférences / Exposition

A3P EndoToxin Day

Suite au succès de la 1ère édition de la Journée Endotoxines, A3P prépare la prochaine édition qui aura lieu le mercredi 4 octobre à Lyon

Une nouvelle journée, associant conférences et table ronde, aura lieu pour couvrir et débattre des thèmes suivants :
- LER (Low Endotoxin Recovery)
- Dispositifs médicaux et les endotoxines
- Nettoyage en place et Assurance de stérilité

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