Box 2 QbD implementation within FDA

Implementation of QbD has started in all three of the review programs in the Office of Pharmaceutical Science: ONDQA, OGD and OBP. Each office is using the concepts of ICH's Q8, Q9 and Q10 guidelines to lay the foundation of the changing review processes. All three offices are implementing at different paces because of the differences in complexity of products and review practices. In the end, all are expected to have successfully implemented QbD.

ONDQA has established a new, pharmaceutical quality assessment system. The new system emphasizes QbD in the evaluation of critical aspects of pharmaceutical quality, with a strong focus on manufacturing science, integration of review and inspection functions and use of modern statistical methodologies. The organization has been restructured to implement the new review process and also to better utilize staff. ONDQA has also successfully implemented a pilot program to determine what information is relevant in applications to demonstrate the key QbD concepts: knowledge of drug substance and drug product and understanding of the respective processes. ONDQA has been the forerunner in implementing the concepts of QbD.

OGD has implemented a QbR process that sets forth important scientific and regulatory review questions that can be answered by the sponsor in the applications. The answers to these questions help the reviewer determine if the product is of high quality and what the level of risk associated with the manufacture and design of the particular product is. Through this process, the manufacturers and FDA reviewers are able to ascertain whether a product is meeting the concepts of QbD. As of June 2007, OGD had received over 400 applications in the QbR format. OGD is in the process of evaluating these applications to determine the effectiveness of the QbR format. The main benefits of the QbR system are to assure product quality through design and performance-based specifications, facilitate continuous improvement and reduce CMC supplements, enhance the quality of CMC reviews through standardized review questions and reduce CMC review time when applicants submit a quality overall summary that addresses the QbR questions[13].

OBP is also in the process of implementing QbD. In many ways, many of the elements of QbD were already imbedded in OBP's regulatory process. It has been a little more difficult to begin to fully implement the concepts of QbD because of the complexity of the biotech products. To determine the relevant QbD information that needs to be included in BLAs and NDAs for biotech products, the FDA has just issued a notice for a pilot program for biotech products[14], similar to the program at ONDQA. It is anticipated that they will start receiving applications with more concrete product and process understanding in the very near future. All three programs will continue to move forward in implementing QbD. There is still much to be learned but the benefits are worth the efforts.

on product quality, and that the process be continually monitored and updated to assure consistent quality over time. As stated in a recent guidance from FDA[12], "Quality by design means designing and developing manufacturing processes during the product development stage to consistently ensure a predefined quality at the end of the manufacturing process." This is seen in Figure 2, which illustrates the different phases during the life cycle of a pharmaceutical process: define, design, characterize, validate, and monitor and control. The final link between "monitor and control" and "define" represents process changes that are initiated based on process improvement opportunities identified during process monitoring or introduced otherwise to improve process performance or robustness. Changes originating in this manner would again go though the cycle illustrated in Figure 2.

The concept of 'design space' is gaining popularity as a tool for implementation of QbD for pharmaceutical products. ICH guidance Q8 (ref. 10) defines design space as "the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post-approval-change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval." Though design space has primarily been used in the context of pharmaceutical processes, it can also be applied to represent the clinical and product-quality aspects of a product. Figure 3 illustrates the interdependency between the clinical, product and process design space.

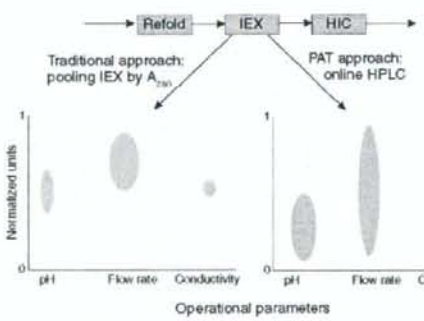

Figure 1 Case study in which IEX step removes impurity 1 to below drug substances specifications. In the traditional approach in which column is pooled by A_{280}, underperformance at the IEX step could result in lot rejection. With the PAT approach, online HPLC allows maintenance of pool quality despite underperformance at the IEX step and achieves a broader process design space. Implementation of PAT control scheme is likely to result in more flexibility during manufacturing. HIC, hydrophobic interaction chromatography.

Defining clinical design space. The concept of clinical design space can be used to quantify the clinical experience with a product. This would be in the form of a multidimensional design space, with each CQA serving as a dimension. The size of the clinical design space for a given product will depend on the number of manufactured lots put in the clinic, process capability, availability of applicable data from other similar products and finally the extent of product heterogeneity that has been introduced during the clinical trials. The last point is noteworthy because although purposely introducing product

heterogeneity to broaden clinical design space should be given consideration, patient safety should not be jeopardized. Process capability will determine the variability observed in the manufactured lots, which will then directly affect the clinical design space.

Figure 3 presents an illustration of clinical design space for two products, X and Y. Product X represents the case of a small clinical trial where only a single lot was used in the clinical study, whereas product Y represents a large clinical trial with five lots used in the clinical trial. The clinical design space is much larger for Y owing to the heterogeneity in product quality introduced into the clinic via use of multiple lots (Fig. 3a).

The clinical design space is expected to be limited in the early phases of clinical development when only a few lots have been introduced into the clinic but then would grow as the product reaches a more advanced stage of product development and more clinical data become available.

Defining product design space. The concept of design space can also be extended to quantify product quality. Similar to the clinical design space, the product design space could be represented as a multidimensional design space with each CQA serving as a dimension. It will be documented in the regulatory filing in the form of in-process, drug substance and drug product specifications and would define the acceptable variability in CQA.

Figure 3b illustrates product design space for the two products, X and Y. As the impact of CQA on safety and efficacy of the product increases (from CQA1 to CQA4), the product design space grows smaller and more similar to the clinical design space for the respective CQA. Because CQA4 is known to have a very significant impact on safety or efficacy of the product, the approved product design space is the same as the clinical design space. This could be also the case if the impact of CQA4 on product safety or efficacy is poorly understood or is unknown. In contrast, if it is well understood that CQA1 does not have a significant impact on the safety or efficacy of the drug product, it is likely that a large product design space would be approved in comparison to the clinical experience with the molecule (the purple and pink ellipses in Figure 3b).

Thus, it follows that improvement in our understanding of the linkage between the CQA and the clinical performance of the product via clinical and nonclinical studies will result in the practitioner being able to claim a broader product design space. Similar to the case of clinical design space, the product design space is much larger for Y due to the larger clinical space for product Y, which in turn was a result of the larger heterogeneity in product quality introduced into the clinic by the use of multiple lots (Fig. 3).

Defining process design space. The concept of process design space is perhaps the most well understood of all three in the pharmaceutical and biotech industry[6]. Once the acceptable variability in CQAs has been established in the form of the product design space, process characterization studies can be used to define the acceptable variability in process parameters[6,22] (Fig. 4). Operating within these acceptable ranges, the combination of which will ultimately define the process design space, provides the 'assurance of quality'. The acceptable ranges are documented in the regulatory filing.

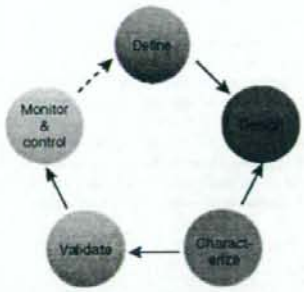

Figure 2 Illustration of the different steps in development of a pharmaceutical product.

The size of the process design space will depend on that of the product design space. This dependency is illustrated in Figure 3c. Similar to the clinical and product design spaces (Fig. 3a,b), the process design space (represented by the ranges for process parameters; pH, flow rate, conductivity) will be much larger for Y than X.

Box 3 A growing literature

Although QbD has been implemented more fully in small-molecule manufacture (e.g., application of the PAT concept to the crystallization process[15] or to freeze-dried parenteral drugs[16]), the literature on the use of QbD in biopharmaceuticals is much smaller. Cook *et al.*[17] published a case study involving design of experiments to identify key and critical process parameters and their targets for a hydrophobic interaction chromatography used in a monoclonal antibody (mAb) purification process. Developing such a 'process design space' is presented in the context of time and resource limitations at a contract manufacturing organization. One of us (A.S.R.) was an author[18] on a case study involving a cell-culture process, multivariate data analysis (MVDA) to support some of the key activities required for successful manufacturing of biopharmaceutical products, including scale-up, process comparability, process characterization and fault diagnosis (for a review of how different biotech companies are exploiting MVDA to solve problems encountered in biotech processing, see ref. 19.) More recently, the group of A.S.R. (ref. 20) has examined application of MVDA to root cause analysis, enabling the identification of scale-up differences and parameter interactions that adversely affect cell culture process performance. We have also successfully used MVDA as a diagnostic tool to identify the root cause of issues encountered during scale-up of a cell culture step and in the design of experimental conditions to demonstrate and correct it. Process parameters and their interactions that adversely affect cell culture performance and product attributes were successfully identified. MVDA is also an effective approach for collating process knowledge and increasing process understanding.

In other work, A.S.R. and collaborators have examined the feasibility of creating and implementing control schemes that enable real-time and product quality–based decisions for commonly used biotech unit operations including protein refolding, process chromatography and ultrafiltration/diafiltration[5]. In all cases, it was concluded that it is possible to design control schemes that rely on measurement of product CQAs and enable real-time decisions. Once the design space for a particular manufacturing process has been defined, it can be continually reassessed and changed, as appropriate[6]. In addition, A.S.R.'s group has published a PAT application involving use of a commercially available online HPLC system for real-time pooling of process chromatography columns[9]. This demonstrated the feasibility of online-HPLC for analysis and its ability to facilitate real-time decisions for column pooling based on product-quality attributes. Thus, the quality systems approach allows continuous improvement of the manufacturing process[12,21].

This translates to greater manufacturing flexibility for product Y when engaging in activities, such as equipment changes, technology transfer, process improvements and other process changes during the life cycle of a pharmaceutical product. It is likely that such changes can be made within the approved process design space for product Y, without requiring regulatory approval. Figure 3 illustrates a key aspect of the QbD paradigm in that upfront planning and investment in establishing broader clinical, product and process design spaces will translate to cost savings during the life cycle of the product.

Case studies from biologics manufacture

Figure 5 illustrates some of the key steps that are taken for implementation of QbD for a biotech product. The steps are in alignment with the recommendations in the ICH guidance Q8 and ICH guidance Q8 Annex documents[9]. Some of these steps are topics of ongoing discussion between the industry and the regulatory agencies and for these we provide a high level overview below. For steps where sufficient clarity exists, we provide a more in depth discussion along with real-life examples. We also highlight the differences between what we have done traditionally and what we would do differently under the QbD paradigm.

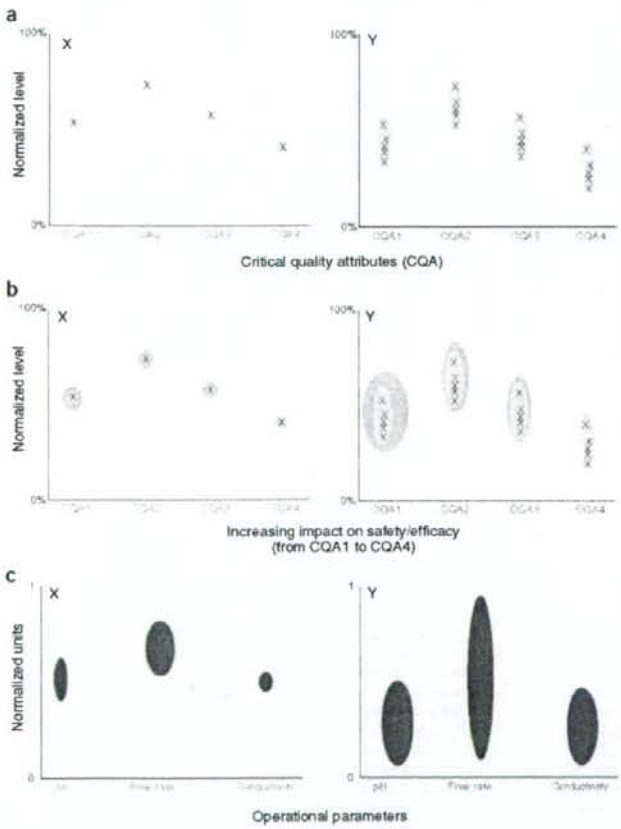

Figure 3 The dependencies among clinical (purple), product (pink) and process (green) design spaces for products X and Y. (a) Impact on the clinical design space of heterogeneity in product quality introduced in the clinic for case X (left, small clinical trials, single lot in clinic) and case Y (right, large clinical trials, five lots in clinic). (b) Impact on product design space of clinical design space and the level of understanding of impact of each CQA on safety and efficacy of products X (left) and Y (right). (c) Impact on process design space of product design space and the impact of each operational parameter on CQA for products X (left) and Y (right).

Identifying target product profile (TPP). TPP has been defined as a "prospective and dynamic summary of the quality characteristics of a drug product that ideally will be achieved to ensure that the desired quality, and thus the safety and efficacy, of a drug product is realized"[10]. This includes dosage form and route of administration, dosage form strength(s), therapeutic moiety release or delivery and pharmacokinetic characteristics (e.g., dissolution and aerodynamic performance) appropriate to the drug product dosage form being developed and drug product-quality criteria (e.g., sterility and purity) appropriate for the intended marketed product[10]. The concept of TPP in this form and its application is novel in the QbD paradigm.

Identifying CQAs. Once TPP has been identified, the next step is to identify the relevant CQAs. A CQA has been defined as "a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality"[10]. Identification of CQAs is done through risk assessment as per the ICH guidance Q9 (ref. 11; Fig. 5). Prior product knowledge, such as the accumulated laboratory, nonclinical and clinical experience with a specific product-quality attribute, is key in making these risk assessments. Such knowledge may also include relevant data from similar molecules and data from literature references. Taken together, this information provides a rationale for relating the CQA to product safety and efficacy. The outcome of the risk assessment would be a list of CQAs ranked in order of importance. Use of robust risk assessment methods for identification of CQAs is novel to the QbD paradigm.

Defining product design space. After CQAs for a product have been identified, the next step is to define the product design space (that is, specifications for in-process, drug substance and drug product attributes). These specifications are established based on several sources of information that link the attributes to the safety and efficacy of the product, including, but not limited to, the following:

- Clinical design space
- Nonclinical studies with the product, such

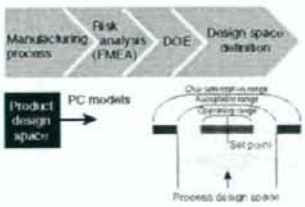

Figure 4 The creation of process design space from process characterization studies and its relationship with the characterized and operating space. The operating range constitutes the operating ranges defined in the manufacturing procedures. The characterization range is the range examined during process characterization. The acceptable range is the output of the characterization studies and defines the process design space. Adapted from ref. 6.

as binding assays, *in vivo* assays and *in vitro* cell-based assays
- Clinical and nonclinical studies with similar platform products
- Published literature on other similar products
- Process capability with respect to the variability observed in the manufactured lots

The difference between the actual experience in the clinic and the specifications set for the product would depend on our level of understanding of the impact that the CQA under consideration can have on the safety and efficacy of the product. For example, taking host cell proteins as a CQA, it is common to propose a specification that is considerably broader than the clinical experience. This is possible because of a greater ability to use data from other platform molecules to justify the broader specifications. On the other hand, in the case of an impurity that is unique to the product, the specifications would rely solely on clinical and nonclinical studies.

In QbD, an improved understanding of the linkages between the CQA and safety and efficacy of the product is required. QbD has brought a realization of the importance of the analytical, nonclinical and animal studies in establishing these linkages and has led to the creation of novel approaches[25–27].

Defining process design space. The overall approach toward process characterization involves three key steps[22–28]. First, risk analysis is performed to identify parameters for process characterization. Second, studies are designed using design of experiments (DOE), such that the data are amenable for use in understanding and defining the design space. And third, the studies are executed and the results analyzed to determine the importance of the parameters as well as their role in establishing design space.

Failure mode and effects analysis (FMEA) is commonly used to assess the potential degree of risk for every operating parameter in a systematic manner and to prioritize the activities, such as experiments, necessary to understand the impact of these parameters on overall process performance[29]. A team consisting of representatives from process development, manufacturing and other relevant disciplines performs an assessment to determine severity, occurrence and detection. The severity score measures the seriousness of a particular failure and is based on an estimate of the severity of the potential failure effect at a local or process level and the potential failure effect at end product use or patient level. Occurrence and detection scores are based on an excursion (manufacturing deviation) outside the operating range that results in the identified failure. Although the occurrence score measures how frequently the failure might occur, the detection score indicates the probability of timely detection and correction of the excursion or the probability of detection before end product use. All three scores are multiplied to provide a risk priority number (RPN) and the RPN scores are then ranked to identify the parameters with a high enough risk to merit process characterization. **Figure 6** illustrates the FMEA outcome for a process chromatography step in a biotech process. RPN scores are calculated and operating parameters with an RPN score >50 are characterized using a qualified scaled-down model. For the case study presented here, these include gradient slope, temperature, flow rate, product loading, end of pool collection, buffer A pH, start of pool collection, volume of wash 1, buffer B pH, buffer C pH and bed height. Process characterization focused on parameters such as temperature, that have a high impact on the process (severity = 6), occur frequently in the manufacturing plant (occurrence = 6) and are difficult to quickly correct if detected (detection = 7). In contrast, parameters such as equilibration volume, with a low impact on the process (severity = 3), low occurrence (occurrence = 2) and a limited ability to detect and correct (detection = 5), were not examined in process characterization.

Because most biotech unit operations are known to be influenced by multiple process parameters, a two-phase study is often the most efficient and effective[27,28]. For the case study presented here, screening is first performed to identify the process parameters that had the most impact on product quality (% purity) and process consistency (% recovery). Nine of the eleven parameters identified by the FMEA are examined. These include: gradient slope, temperature, flow rate, product loading, end of pool collection, buffer A pH, start of pool collection, bed height and buffer B pH. Buffer C pH and volume of wash 1 are examined separately in different studies.

Temperature, buffer A pH and buffer B pH are found to affect % recovery and % purity in a manner that is significant both statistically ($P < 0.05$) and in magnitude (>20%, recovery; >5%, purity; **Fig. 7a**). Product loading, flow rate and bed height are found to affect % recovery (but not % purity) in a manner that is significant both statistically and in magnitude. All six of these parameters are further examined for their interactions via a DOE study. **Figure 7b** presents the outcome of one such DOE study that examined the effect of these process parameters on % purity. It is seen that temperature and buffer A pH are found to affect % purity in a manner that is significant both statistically

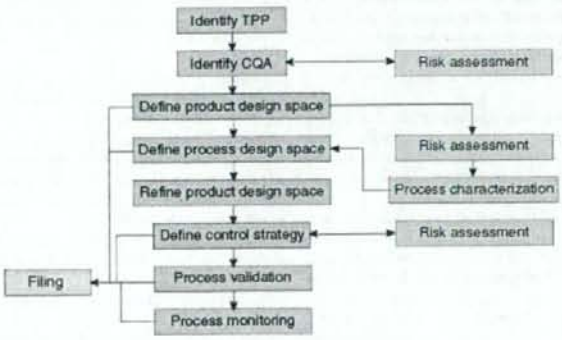

Figure 5 Key steps in implementation of QbD for a biotech product.

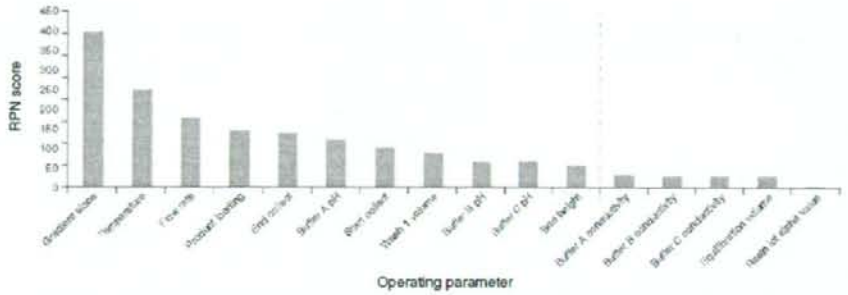

Figure 6 Pareto chart showing RPN scores for the operating parameters for a chromatography step in a biotech process. Parameters that had RPN scores higher than the cutoff (RPN = 50) were further examined in process characterization.

and in magnitude. On the basis of these results, temperature and buffer A pH are identified as critical process parameters. In a separate study, impact of process parameters on % recovery is examined and based on the results, buffer B pH, product loading, flow rate and bed height are identified as key process parameters. At the conclusion of the process-characterization studies, data are analyzed to calculate the acceptable ranges for the key and critical process parameters. These acceptable ranges together defined the design space, as presented in **Table 2**. It can also be seen that the operating space, as defined by the operating ranges, is well nested inside the design space indicating robustness of the process step (**Fig. 4**).

Although FMEA and DOE are not new concepts for the development of biotech processes, linking the establishment of design space to the relevant CQA is novel. For example, a chromatography step that has a direct impact on several CQAs and a direct bearing on whether the final drug product meets specifications would be expected to undergo a more thorough process characterization and examination of a larger process design space. In contrast, an ultrafiltration/diafiltration step that is robust and has no direct influence on any CQA may require relatively limited process characterization.

Defining control strategy. Control strategy is defined as "a planned set of controls, derived from current product and process understanding that assures process performance and product quality"[12]. The control strategy in the QbD paradigm is established via risk assessment that takes into account the criticality of the CQA and process capability (**Fig. 5**). The control strategy can include the following elements: procedural controls, in-process controls, lot release testing, process monitoring, characterization testing, comparability testing and stability testing. It is worth noting that the use of risk assessment in creating the control strategy is unique to the QbD approach.

Process validation. An enhanced understanding of the manufacturing process and an expanded process design space should provide more manufacturing flexibility during process validation[14]. Because the process design space "assures quality" of the drug product, these limits should also provide the basis of the validation acceptance criteria. The limits that establish the acceptable variability in product-quality and process-performance attributes would also serve as the process validation acceptance criteria (**Fig. 4**). Once the process design space has been created, process validation becomes an exercise to demonstrate (i) that the process will deliver a product of acceptable quality if operated within the design space and (ii) that the small and/or pilot scale systems used to establish the design space accurately model the performance of the manufacturing scale process. Thus, in the QbD paradigm, unanticipated manufacturing excursions that remain within the process design space should not jeopardize the success of the validation exercise.

Regulatory filings. After the process design space has been established and validated, the regulatory filing would include the acceptable ranges for all key and critical operating parameters that define the process design space in addition to a more restricted operating space typically described for drug products. The filing would also include the refined product design space, description of the control strategy, outcome of the validation exercise and plan for process monitoring (**Fig. 5**). In the QbD paradigm, the filing could also include protocols (e.g., comparability protocols or expanded change protocols) that would allow future flexibility in process changes with respect to pre-approved criteria that have been agreed upon between the applicant and the agency.

Process monitoring, life-cycle management and continuous improvement. After approval, CQAs would be monitored to ensure that the process is performing within the defined acceptable variability that served as the basis for the filed process design space[6]. The primary benefit of an expanded process design space would be a more flexible approach by regulatory agencies. In the QbD paradigm, process changes within the design space will not require review or approval[3]. Therefore, process improvements during the product life cycle with regard to process consistency and throughput could take place with fewer post-approval submissions. In addition to regulatory flexibility, the enhanced understanding of the manufacturing process would allow more informed risk assessment as per ICH Q9 regarding the affects of process changes and manufacturing deviations (excursions) on product quality[13]. As manufacturing experience grows and opportunities for process improvement are identified, the operating space could be revised within the design space without the need for post-approval submission.

Over the lifetime of a product, process changes may be required to be made and may require process characterization, validation and filing of the changes to the approved process design space (**Fig. 2**). The quality system needs to provide adequate oversight during QbD implementation to changes that will not go through regulatory approval[12]. Robustness of the quality system would need to be demonstrated with respect to the following four elements: process performance/product-quality monitoring; preventative/corrective action, change management and management review of process performance and product quality.

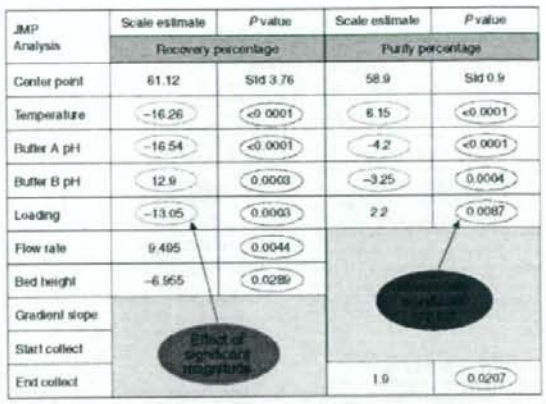

Figure 7 Results from the process characterization studies performed at small scale. (a) Screening study involving examination of nine operational parameters for a chromatography step in a biotech process. Six of the operational parameters were identified for further study via DOE: temperature, buffer A pH, buffer B pH, product loading, flow rate and bed height. Std, standard deviation. (b) Results from the DOE study showing the effect of different operational parameters on % purity for a chromatography step in a biotech process. Temperature and buffer A pH were found to have an effect that is significant both statistically and in magnitude. The green ellipses indicate parameters that had an effect of significant magnitude (>20% for recovery and >5% for purity). The red ellipses indicate parameters that had a statistically significant impact ($P < 0.05$). Buffer B pH * load rate denotes a first order interaction between these two parameters. Term "t ratio" stands for the test of significance and compares the difference with the standard error of the difference.

Discussion

The benefits to FDA with the implementation of QbD are tremendous. These include, but are not limited to, enhancing the scientific foundation of product review; better coordination across review, compliance and inspection; improving information in regulatory submissions; more consistency of regulatory decision making; improving quality of review (establishing a quality management system for CMC); more flexibility in decision making; ensuring decisions are made on science and not on empirical information; involving various disciplines in decision making; and using resources to address higher risks. It is important for FDA to take advantage of QbD to improve efficiencies in its operations as to help ensure the quality of marketed products.

Likewise, QbD provides numerous advantages to industry[21]. It ensures better design of products with fewer problems in manufacturing, reduces the number of manufacturing supplements required for post-market changes, relies more on process, and understanding and mitigation of risk, allows implementation of new technology to improve manufacturing without regulatory scrutiny, enables possible reduction in overall costs of manufacturing resulting in less waste, ensures efficient review and thus reduced deficiencies resulting in quicker approvals, enables continuous improvements in products and manufacturing, provides better understanding of how active pharmaceutical ingredients and excipients affect manufacturing and relates manufacturing processes to the clinic during design, thereby providing a better overall business model.

With all changes come opportunities and challenges. The opportunities under QbD are obvious. The main opportunity is to improve the overall system—ensuring that the system is efficient, agile and flexible. QbD is also designed to increase manufacturing efficiencies and to help reduce costs associated with manufacturing based on fewer product rejections and less waste. QbD will also help to build a stronger scientific knowledge base for all products and better overall interaction of the agency with industry on scientific issues. It is also an opportunity to incorporate risk management approaches to the regulatory processes. Overall, it would be important to demonstrate robustness of the quality system with respect to process performance and product quality monitoring, corrective/preventative action, change management, and management review of process performance and product quality.

There are still many challenges as FDA promotes the concepts of QbD. We are at the beginning of the process of developing an overall QbD approach. One of the main challenges is terminology. The agency and industry need to agree on definitions of the various terms used (e.g., design space, CQA). FDA is also in the process of determining the appropriate relevant information that needs to be included in submissions as well as how we will handle post-market management (e.g., a plan for manufacturing changes that will not require supplements). The biggest challenge, though, is how to handle products that do not incorporate the tenets of QbD, such as legacy products that were not approved using QbD. For such products, FDA will still need to maintain a dual system that allows flexibility.

Table 2 Design space for case study involving characterization of a process chromatography step

Process parameter	Categorization	Operating range[a]	Acceptable range[b]
Temperature	Critical	10 ± 1 °C	10 ± 3 °C
Buffer A pH	Critical	6.2 ± 0.1	6.2 ± 0.2
Buffer B pH	Key	6.2 ± 0.1	6.2 ± 0.2
Flow rate	Key	0.06 ± 5% CV/min	0.06 ± 10% CV/min
Product loading	Key	8 AU/ml	7–9 AU/ml
Bed height	Key	18 ± 1 cm	18 ± 3 cm

[a]Operating ranges constitute the operating space for the process step. [b]Acceptable ranges define the process design space for the step. AU, arbitrary units.

Another major challenge is training. It will be important for FDA and the industry to provide training programs to enable appropriate implementation of QbD.

Industry, academia and FDA need to work together to pursue the opportunities, overcome the challenges and realize the benefits that QbD implementation has to offer. The QbD pilots of ONDQA and the OBP will help assess industry's capabilities and provide information that supports the concepts of QbD. Experience with the question-based review (QbR) process at the Office of Generic Drugs (OGD; Box 2) process will also help determine whether the questions adequately meet our needs for information to better understand QbD for generic drug products.

At the present time, it is clear that the complexity of biotech products and processes makes complete characterization impractical. Although there are similarities in the manufacturing processes for both small and large molecules, biologics present some unique issues that will now need to be addressed in order for implementation of QbD to move forward. However, both industry and regulators agree on the benefits of the QbD approach as well as the fact that full implementation will be a multistep process involving discussion within industry and between industry and regulators.

The planned pilot program of the OBP for biotech products will be useful in resolving these issues. Case studies, such as those presented here, will also serve as useful tools in gaining the common ground and defining best practices in the various functions: R&D, quality assurance, manufacturing and regulatory affairs. They can be used to advise and train personnel both at companies and the FDA in the concepts of QbD and how they can be used effectively. FDA will also continue its role in global harmonization, specifically through its involvement with ICH. Without such harmonized global regulation, the agency's goals for modernization of quality will be difficult to achieve.

ACKNOWLEDGMENTS

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DISCLAIMER

This article reflects the current thinking and experience of H.W.; however, it is not an FDA policy document nor should it be used in lieu of regulations, published FDA guidance or direct discussions with the agency. No official support or endorsement of this article by the Food and Drug Administration is intended or should be inferred.

1 US Food and Drug Administration. FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products (FDA, Rockville, MD, April 1996). <http://www.fda.gov/cder/Guidance/compare.htm>
2 US Food and Drug Administration. Revised guidance for industry: providing regulatory submissions to the Center for Biologics Evaluation and Research (CBER) in electronic format – biologics marketing applications (Biologics License Application (BLA), Product License Application (PLA)/ Establishment License Application (ELA) and New Drug Application (NDA)) (FDA, Rockville, MD, November 1999) <http://www.cfsan.fda.gov/~dms/opagu3.html>
3 US Food and Drug Administration. Pharmaceutical cGMPs for the 21st century: a risk-based approach (FDA, Rockville, MD, August 2002) <http://www.fda.gov/oc/guidance/gmp.html>
4 US Food and Drug Administration. PAT guidance for industry—a framework for innovative pharmaceutical development, manufacturing and quality assurance (US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, Office of Regulatory Affairs, Rockville, MD, September, 2004).
5 Rathore, A.S., Sharma, A. & Chilkin, D. *BioPharm Int.* **19**, 48–57 (2006).
6 Rathore, A.S., Branning, R. & Cecchini, D. *BioPharm Int.* **20**, 36–40 (2007).
7 Munson, J., Stanfield, C.F. & Gupal, B. *Curr. Pharm. Anal.* **2**, 405–414 (2006).
8 Gnoth, S., Jenzsch, M., Simutis, R. & Lubbert, A. *J. Biotechnol.* **132**, 180–186 (2007).
9 Rathore, A.S., Yu, M., Yadavalli, S. & Sharma, A. *Biotechnol. Bioeng.* **100**, 306–316 (2008).
10 US Food and Drug Administration. Guidance for industry: Q8 pharmaceutical development, US Department of Health and Human Service (FDA, Rockville, MD, May 2006).
11 US Food and Drug Administration. Guidance for industry: Q9 quality risk management, US Department of Health and Human Service (FDA, Rockville, MD, June 2006).
12 US Food and Drug Administration. guidance for industry: Q10 quality systems approach to pharmaceutical cGMP regulations (FDA, Rockville, MD, September 2006).
13 Yu, L.X. *Pharm. Res.* **25**, 781–791 (2008).
14 Office of Biotechnology Products, US Food and Drug Administration. Notice of pilot program for submission of quality information for biotechnology products in the Office of Biotechnology Products, Food and Drug Administration, docket number FDA-2008-N-0355 (FDA, Washington, DC, 2008) <http://www.fda.gov/OHRMS/DOCKETS/98fr/FDA-2008-N-0355-n.pdf>
15 Yu, L.X. *et al. Adv. Drug Deliv. Rev.* **56**, 349–369 (2004).
16 Neil, S.L. & Searles, J.A. *BioPharm Int.* **21**, 44–51 (2008).
17 Cook, S., Patton, K.A. & Bazemore, L.R. *BioPharm Int.* **20**, 28–35 (2007).
18 Kirdar, A.O., Conner, J.S., Baclaski, J. & Rathore, A.S. *Biotechnol. Prog.* **23**, 61–67 (2007).
19 Johnson, R. *et al. BioPharm Int.* **20**, 130–144 (2007).
20 Kirdar, A.O., Green, K.D. & Rathore, A.S. *Biotechnol. Prog.* **24**, 720–726 (2008).
21 Kozlowski, S. & Swann, P. *Adv. Drug Deliv. Rev.* **58**, 707–722 (2006).
22 Harms, J., Wang, X., Kim, T., Yang, J. & Rathore, A.S. *Biotechnol. Prog.* **24**, 655–662 (2008).
23 Stein, K.E. *Trends Biotechnol.* **15**, 88–90 (1997).
24 Mire-Sluis, A.R. *et al. J. Immunol. Methods* **289**, 1–16 (2004).
25 Klakamp, S.L., Lu, H., Tabrizi, M., Funelas, C. & Rokkos, L.F. *Anal. Chem.* **79**, 8176–8184 (2007).
26 Barrett, Y.C., Ebling, W., Pieniazek, H., Ballheimer, J. & Seffert, D. *J. Pharm. Biomed. Anal.* **44**, 938–946 (2007).
27 Gupta, S. *et al. J. Immunol. Methods* **321**, 1–18 (2007).
28 Seely, J. in *Process Validation in Manufacturing of Biopharmaceuticals* (eds. Rathore, A.S. & Sofer, G.) 31–68 (Taylor & Francis, Boca Raton, FL, 2005).
29 Seely, R.J. & Houry, J. in *Process Validation in Manufacturing of Biopharmaceuticals* (eds. Rathore, A.S. & Sofer, G.) 13–30 (Taylor & Francis, Boca Raton, FL, 2005).

資料2

PharmaManufacturing.com

Conformia and Leading Biotech Manufacturers To Collaborate in Unprecedented Effort of Advancing Application of Quality By Design Paradigm in Biotech Environment

PharmaManufacturing.com
07/28/2008

Conformia, the market leader for product/process lifecycle management (PPLM) solutions for the pharmaceutical and biotech industries, on July 28 announced the formation of the Chemistry Manufacturing and Controls (CMC) Biotech Working Group, an expansion of the CMC-IM Working Group formed in July 2007. The group, which includes seven leading biotech industry players, Amgen, Genentech, Abbott Bio MedImmune (AstraZeneca), GlaxoSmithKine Bio, Eli Lilly Bio and Pfizer Bio, is designed to enable the creation of a fictitious but realistic example of a monodonal antibody whose development program embraces the ICH quality vision (ICH Q8, Q9, and Q10). The goal of the case study is to provide a series of examples that can be used to assist companies in interpreting the ICH quality guidelines and applying them to real life scenarios.

"The ICH documents provide a science based approach for assuring quality in the manufacture of medicinal products," said Dr. Kenneth B. Seamon, Institute of Biotechnology , University of Cambridge and co-facilitator of the CMC Biotech Working Group. "The CMC Biotech Working Group will provide additional detail on approaches for implementing the harmonized guidance that will be transparent based on the best science. This will provide significant business benefits to the industry as well as facilitate a more innovative and comprehensive approach for national authorities to review and assure the quality of biopharmaceuticals."

"Pfizer is pleased to work with Conformia on this important initiative," said James Spavins, vice president, regulatory CMC/QA at Pfizer, Inc. "We look forward to collaborating with the other companies in the CMC Biotech Working Group to advance discussion and create examples of Quality by Design (QdB) for the Biotech Industry."

This new collaboration, which will include Amgen, Genentech, Abbott, MedImmune, GlaxoSmithKine, Eli Lilly, and Pfizer, is built on Conformia's experience and leadership conducting the development of a mock case study for large pharmaceuticals (called ACE) and the formation of the CMC-IM Working Group launched in 2007. The CMC Biotech Working Group is an extension of this not-for-profit industry effort to advance understanding of the ICH quality vision across the globe.

Conformia has been a CRADA partner of the FDA since 2006 and has successfully launched a workshop series centered on case study discussion. The purpose of Conformia's CRADA is to identify how the core principles of ICH can be applied in pharmaceutical development and to better understand where the pharmaceutical firms face challenges as they bring new drugs to market and attempt to maintain highest levels of quality.

The Conformia CRADA findings from the pilot study indicated a significant unmet need in the availability of examples for

biotech product/process development. Furthermore, according to ICH, the top 30 companies, by fully implementing the ICH guidelines, stand to save a total of $8 billion a year. The CMC Biotech Working Group will advance collective understanding to help accelerate adoption of these guidelines for a biotech development and the production environment.

"We can now leverage what we learned previously through creation of the ACE case study, which focused on a small molecule drug development paradigm embracing the ICH quality vision, and build upon that understanding in the biotech arena," said Anjali Kataria, founder of Conformia and principal investigator of the CRADA with the FDA. "The lessons we learned from our CRADA recognized a strong desire for better industry/agency communication as well as a need for collaboration across the board to apply the QdB paradigm to the biotech industry."

Kataria continued by explaining, "The outputs of the CMC Biotech Working Group will not only be available for the industry, but also for regulators across the globe who seek to better understand how the concepts of the ICH quality vision might be applied in day-to-day practice inside firms. This will undoubtedly help bring the level of discussion between regulators and the industry to a deeper understanding of each other."

"The level of commitment from all the companies participating in the CMC Biotech Working Group demonstrates a singular intention to facilitate understanding of the new quality paradigm," said Dr. John Berridge, quality consultant to Conformia and working group co-facilitator. "Together, senior leaders from many top pharmaceutical and biotech companies will debate and share views on the core principles to create a valuable case study of a monoclonal antibody for pharmaceutical development teams to reference as they implement the new ICH guidelines."

About Conformia

Conformia Software provides product/process lifecycle management (PPLM) software solutions to pharma and biotech enabling design, development and transfer. These solutions enable companies in highly regulated industries to save significant time and money through major improvements in product/process development operations. Specifically, Conformia can help companies improve product development cycle times (speed), reduce risks and decrease costs. For more information and to download the ACE Case Study, visit www.conformia.com.

資料3

European Medicines Agency
Pre-authorisation Evaluation of Medicines for Human Use

London, 18 December 2008
EMEA/CHMP/BWP/157653/2007

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON DEVELOPMENT, PRODUCTION, CHARACTERISATION AND SPECIFICATIONS FOR MONOCLONAL ANTIBODIES AND RELATED PRODUCTS

DRAFT AGREED BY BIOLOGICS WORKING PARTY	18 April 2007
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	24 May 2007
END OF CONSULTATION (DEADLINE FOR COMMENTS)	30 November 2007
AGREED BY BIOLOGICS WORKING PARTY	12 November 2008
ADOPTION BY CHMP	18 December 2008
DATE FOR COMING INTO EFFECT	1 July 2009

This guideline replaces the guideline on "*Production and quality control of monoclonal antibodies*" (3AB4A)

This guideline replaces the quality requirements for monoclonal antibodies set forth in the guideline on "*Radiopharmaceuticals based on monoclonal antibodies*" (3AQ21A)

KEYWORDS	*Monoclonal antibody, recombinant proteins, quality, characterisation, specification, hybridoma*

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel (44-20) 74 18 84 00 Fax (44-20 74 18 84 16)
E-mail mail@emea.europa.eu http://www.emea.europa.eu

GUIDELINE ON DEVELOPMENT, PRODUCTION, CHARACTERISATION AND SPECIFICATIONS FOR MONOCLONAL ANTIBODIES AND RELATED PRODUCTS

TABLE OF CONTENTS

1. INTRODUCTION

This guideline lays down quality requirements for monoclonal antibodies.

Monoclonal antibodies are immunoglobulins (Ig) with a defined specificity derived from a monoclonal cell line. Their biological activities are characterised by a specific binding characteristic to a ligand (commonly known as antigen), and may be dependent on immune effector function such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

Monoclonal antibodies may be generated by recombinant DNA (rDNA) technology, hybridoma technology, B lymphocyte immortalisation or other technologies (e.g. display technology, genetically engineered animals).

This guideline covers principles and general requirements for development, production, characterisation and specifications for monoclonal antibodies to be used as, or in the production of, human medicinal products.

2. SCOPE

This guideline addresses quality issues for the marketing authorisation of monoclonal antibodies derived from a monoclonal cell line, and intended for therapeutic and prophylactic use (including *ex vivo* application), and *in vivo* diagnostic use.

The principles described in this document apply to monoclonal antibodies used as reagents, as well as monoclonal antibody-related products, such as fragments, conjugates, and fusion proteins. However, their applicability will be determined on a case-by-case basis, based on their specific properties, and may be addressed in specific annexes.

Polyclonal antibodies (fractionated or recombinant) are outside the scope of this guideline, although its principles should be applied where appropriate.

The scope of this guideline does not include:
- Monoclonal antibodies to be used for diagnostic purposes *in vitro*;
- Monoclonal antibodies used in clinical trials. However, the principles described in this document should be taken into account in the production and control of monoclonal antibodies in clinical trials, and their applicability will be determined on a case-by-case basis.

3. LEGAL BASIS

This guideline should be read in conjunction with the introduction and general principles and Part 2 of Annex I of Directive 2001/83/EC, as amended.

This guideline replaces the guideline on "*Production and quality control of monoclonal antibodies*" (3AB4a).

This guideline should be read in conjunction with all other relevant guidelines, especially those pertinent to the production and quality control of rDNA products. Furthermore, reference is made to the Ph. Eur. monograph on "*Monoclonal antibodies for human use*" (2031).

4. MAIN GUIDELINE TEXT

4.1. DEVELOPMENT OF THE MONOCLONAL ANTIBODY

The structure of the monoclonal antibody should be justified with respect to its mechanism of action, biological activity and stability. This justification should at least include discussion on the suitability of the product's immunochemical properties (e.g. affinity, cross-reactivity, isotype, allotype) and the importance and integrity of effector function. Furthermore, the risk of inducing antibody responses in patients should be carefully considered, especially when the product does not have a high homology

with human immunoglobulin, or when potentially immunogenic epitopes are identified in the structure, as it may result in clinical adverse reactions and/or modify the therapeutic potential.

The cell substrate to be used for the production of the monoclonal antibodies should be a stable and continuous monoclonal cell line that has been developed by means of recombinant DNA and/or other suitable technologies. The rationale for selecting the cell substrate should be discussed with regards to its capability to produce the desired product quality, compared to other relevant approaches.

When the cell substrate is obtained by recombinant DNA technology, a description of the expression system used for the production of antibodies should be in accordance with relevant guidelines, especially *"Production and Quality Control of medicinal products derived by recombinant DNA technology"*(3AB1A), and the relevant ICH guidelines Q5A (viral safety), Q5B (expression constructs) and Q5D (cell substrates).

When one or more specific procedures are performed during development, prior to the isolation of the monoclonal cell line, such as cell fusion, viral transformation, gene library of phage display screening, application of *in silico, in vitro* or *in vivo* technologies, these procedures do not need to be described in great detail. However, sufficient information on these procedures should be provided to allow assessment of the identity and purity of the monoclonal cell line, when relevant to the safety and efficacy of the product (e.g. amino acid or post-translational modifications to modulate immunogenicity or effector functions, and information regarding adventitious agents and potential contaminants).

The immortalisation of a human or non-human B-lymphocyte through cell fusion or transformation may be necessary to obtain a stable and continuous monoclonal cell line to be used for antibody production. The choice of using this approach should be cautiously considered and appropriately justified with regards to safety and efficacy.

The use of human B-lymphocytes as parental cell lines raises specific concerns with respect to the transmission of infectious agents, including the agent causing variant Creutzfeldt-Jakob disease (vCJD), as well as other human pathogens. The use of human lymphocytes transformed with Epstein-Barr virus (EBV) raises further concerns due to the presence of infectious EBV.

Hybridoma cell lines obtained through the fusion of human or non-human B-lymphocytes with myeloma cells may be used as cell substrates. The origin and characteristics of the parental cell should be documented, including information on the health history of the donors, the fusion partner used and the materials of human or animal origin to which it has been exposed (e.g. feeder cells and myeloma cells).

4.2. PRODUCTION OF MONOCLONAL ANTIBODIES

4.2.1. General considerations

The manufacturing process should be appropriately described and validated. Validation studies should at least include i) the demonstration that the process is capable of producing product of consistent quality, in line with an appropriately defined control strategy, ii) an evaluation of the process capability (e.g. elimination of process-related impurities, viruses), and iii) the demonstration that each operational unit performs appropriately (e.g. validation of purification column, aseptic filling).

Attention should be focused on the setting of in-process controls (including product quality attributes and process parameters), as well as the drug substance and drug product specifications. These controls should be capable of monitoring relevant quality attributes, such as product-related substances and impurities (e.g. disulfide bond integrity or mismatch, deamidation, oxidation, truncation, aggregates) or process-related impurities (e.g. host cell protein, DNA, protein A, bovine serum and culture media residues), as well as relevant process parameters (e.g. column loads, pH, temperature).

When protein A is used in the purification process, the source of the protein A (e.g. *S. aureus*, recombinant) and its preparation method (e.g. purified using human IgG) should be appropriately documented. Where human IgG has been used in the preparation, it should be demonstrated that the quality of human IgG is suitable for its intended use, especially with regards to viral safety.

4.2.2. Platform manufacturing

The development of processes used for the production of monoclonal antibodies very much depends on the manufacturer's knowledge of the product and manufacturing process.

Some manufacturers have gained considerable experience in the production of monoclonal antibodies, and have developed a production strategy based on similar manufacturing processes (i.e. using a pre-defined host cell, cell culture and purification process). This approach is often referred to as "platform manufacturing".

As for any medicinal product, the manufacturing process of a product that has been developed using a platform manufacturing approach should be appropriately validated at the time of marketing authorisation application. Validation studies should include data derived from the final manufacturing process and site(s) used to produce the product to be commercialised. Nevertheless, when appropriately justified and documented, data derived from other relevant experience may be used to support or reduce the data derived from the final commercial process to be submitted.

Considering that quality attributes are specific for a given product and its manufacturing process, the suitability of analytical methods, and more generally the control strategy, should be specifically demonstrated for the product and process being registered. As a consequence, the suitability of the control strategy, demonstrated to be suitable for the analysis of other product(s) derived from the same platform manufacturing approach, should be carefully re-considered, as it may not be adapted to the product and process being submitted. For instance, process-related impurities, such as host cell proteins (HCP), are highly dependent on the process, and the controls applied for a given product and process may not be suitable for other products using the same platform manufacturing (e.g. different cell substrates derived from a common parenteral cell line, similar culture and purification conditions).

When a change is made to an already authorised process following a platform manufacturing approach, the impact of this change should be specifically evaluated for the concerned product and process. Nevertheless, when appropriately justified and documented, data derived from relevant experience may be used to support or reduce the data derived for the post-changed product and process to be submitted. Furthermore, when several products are derived from a common platform manufacturing process, and modifications (e.g. process optimisation, improvement) are introduced in only one or some of them, the rationale for the harmonisation strategy adopted or for the lack of harmonisation should be discussed.

4.2.3. Viral safety and Transmissible Spongiform Encephalopathy (TSE)

Viral safety aspects of monoclonal antibodies covered by this guideline should comply with ICH Q5A. The scope of this guideline includes monoclonal antibodies derived from hybridoma cell lines or from cells genetically engineered to express a monoclonal antibody. Whenever production of monoclonal antibodies is performed using animals (e.g. engineered animals or harvest from ascites fluid), ICH Q5A should be followed with particular reference to Appendix 1. Source cells (e.g. host cells) should undergo suitable screening for adventitious agents (i.e. extraneous agents and endogenous agents). The choice of viruses for the tests is dependent on the species and tissue of origin of the production cell and the nature of any other biological raw material used in production.

The importance of good studies on the validation of viral reduction is emphasised. The virus-reducing capacity of manufacturing steps should be validated for the submitted product and its manufacturing process according to ICH Q5A. These validation studies are usually performed using intermediates from the specific production process in order to cover potential or unexpected product-specific factors affecting virus reduction. Nevertheless, when appropriately justified and documented, relevant studies

(e.g. derived from a platform manufacturing approach) can also be helpful to establish and evaluate virus-reducing process steps, and thus may help to reduce the number of validation studies to be submitted. Such data may be considered supportive, e.g. for investigation of the potential influence of varying process parameters on virus reduction, performance of columns after multiple production cycles, virus carry-over studies or studies on column sanitisation. In all cases, the manufacturer should justify the relevance of these data for the specific product. A rationale should be provided why prior in-house data can be applied to the new product, e.g. referring to viral reduction data of a particular process step would be possible when the product intermediate at the stage before such a step has comparable biochemical properties and is purified by identical methods. The manufacturer should provide a critical analysis of the manufacturing step for which these supportive in-house data will be applied and on the composition of the respective product intermediate. The analysis should provide confidence in the conclusion that in both cases the established manufacturing step is similar in its capacity to inactivate/remove potential virus contaminants. If the comparison of the step is not entirely convincing, or if the database cannot rule out a product-specific effect on virus reduction capacity, confirmatory runs using product-specific process intermediates are expected.

Where materials of bovine or other TSE-relevant animal species have been used in development or manufacture, the Note for Guidance on "*Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products*" (EMEA/410/01) should be consulted.

4.3. CHARACTERISATION OF MONOCLONAL ANTIBODIES

The monoclonal antibody should be characterised thoroughly. This characterisation should include the determination of physicochemical and immunochemical properties, biological activity, purity, impurities and quantity of the monoclonal antibody, in line with ICH Q6B guideline. At the time of submission, the manufacturer should have established appropriately characterised in-house reference materials which will serve for biological and physicochemical testing of production lots.

4.3.1. Physicochemical characterisation

A physicochemical characterisation program will generally include a determination of the class, subclass, light chain composition (kappa and/or lambda chain) and primary structure of the monoclonal antibody.

The amino acid sequence should be deduced from DNA sequencing and confirmed experimentally by appropriate methods (e.g. peptide mapping, amino acid sequencing, mass spectrometry analysis). The variability of N- and C- terminal amino-acid sequences should be analysed (e.g. C-terminal lysine(s)).

Free sulphydryl groups and disulfide bridges should be determined. Disulfide bridge integrity and mismatch should be analysed.

The carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), the glycosylation site(s) and occupancy should be analysed.

Typically, monoclonal antibodies have one N-glycosylation site on each heavy chain located in the Fc region. The light chain is usually not glycosylated. However, additional glycosylation site(s) in the heavy chains may occur, and thus their presence or absence should be confirmed. Glycan structures should be characterised, and particular attention should be paid to their degree of mannosylation, galactosylation, fucosylation and sialylation. The distribution of the main glycan structures present (often G0, G1 and G2) should be determined.

Higher-order structure of the monoclonal antibody should be characterised by appropriate physicochemical methodologies.

4.3.2. Immunological properties

The immunological properties of the antibody should be fully characterised. Binding assays of the antibody to purified antigens and defined regions of antigens should be performed, where feasible, to determine affinity, avidity and immunoreactivity (including crossreactivity with other structurally homologous proteins). Unintentional reactivity/cytotoxicity for human tissues distinct from the intended target should be documented. Crossreactivity with a range of human tissues should be determined using immunohistochemical procedures (see Annex I). Where appropriate, cross reference to non-clinical and/or clinical section(s) may be made.

The complementary determining regions (CDR) should be identified, unless otherwise justified.

The epitope and molecule bearing the relevant epitope should be defined. This should include a biochemical identification of these structures (e.g. protein, oligosaccharide, glycoprotein, glycolipid), and relevant characterisation studies (amino acid sequence, carbohydrate structure) to the extent possible.

The ability for complement binding and activation, and/or other effector functions should be evaluated, even if the intended biological activity does not require such functions.

4.3.3. Biological activity

The biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological effect) should be assessed by *in vitro* and/or *in vivo* assays as appropriate. The mechanism of action and the importance (or consequences) of the product effector functions with regards to the safety and efficacy of the product should be discussed.

For antibodies where effector function may play a role in the mechanism of action, and/or have an impact on the product safety and efficacy, a detailed analysis of ADCC, cytotoxic properties (e.g. apoptosis), ability for complement binding and activation and other effector functions, including Fc gamma receptor binding activity, and neonatal Fc receptor (FcRn) binding activity should be provided, as appropriate.

4.3.4. Purity, impurity and contaminants

Monoclonal antibodies commonly display several sources of heterogeneity (e.g. C-terminal lysine processing, N-terminal pyroglutamate, deamidation, oxidation, isomerisation, fragmentation, disulfide bond mismatch, N-linked oligosaccharide, glycation), which lead to a complex purity/impurity profile comprising several molecular entities or variants. This purity/impurity profile should be assessed by a combination of orthogonal methods, and individual and/or collective acceptance criteria should be considered for relevant product-related variants.

These methods generally include the determination of physicochemical properties such as molecular weight or size, isoform pattern, extinction coefficient, electrophoretic profiles, chromatographic data and spectroscopic profiles. In addition, suitable methods should be proposed to qualitatively and quantitatively analyse heterogeneity related to charged variants.

Multimers and aggregates should also be appropriately characterised using a combination of methods. The formation of aggregates, sub-visible and visible particulates in the drug product is important and should be investigated and closely monitored on batch release and during stability studies. In addition to the pharmacopoeial test for particulate matter, other orthogonal analytical methods may be necessary to determine levels and the nature of particulates.

Potential process-related impurities (e.g. HCP, host cell DNA, cell culture residues, downstream processing residues) should be identified, and evaluated qualitatively and/or quantitatively, as appropriate.

Contaminants, which include all adventitiously introduced materials not intended to be part of the manufacturing process (e.g. microbial species, endotoxins) should be strictly avoided and/or suitably controlled. Where non-endotoxin pro-inflammatory contaminants, such as peptidoglycan, are suspected, the use of additional testing, such as the monocyte activation test, should be considered.

4.3.5. Quantity

Quantity should be determined using an appropriate physicochemical and/or immunochemical assay.

It should be demonstrated that the quantity values obtained are directly related to those derived using the biological assay. When this correlation exists, it may be appropriate to use measurement of quantity rather than the measurement of biological activity in the product labelling and manufacturing processes, such as filling.

4. 4. SPECIFICATIONS

Specifications are one part of a total control strategy designed to ensure product quality and consistency, and when tested, the product should be in compliance with its specification. Specifications should be set and take into account relevant quality attributes identified in characterisation studies. Selection of tests to be included in the specifications is product specific. The rationale used to establish the acceptable range of acceptance criteria should be described. Acceptance criteria should be established and justified taking into account data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency, data from stability studies and relevant development data, in accordance with ICH Q6B.

4.4.1. Identity

The identity test(s) should be highly specific and should be based on unique aspects of the product's molecular structure and/or other specific properties (e.g. peptide map, anti-idiotype immunoassay, or other appropriate method). Considering the great similarity of the constant domains of different antibodies, more than one test (physicochemical, biological and/or immunochemical) may be necessary to establish identity, and such test(s) should be able to discriminate other antibodies that may be manufactured in the same facility.

4.4.2. Purity and impurities

As noted in the characterisation section, monoclonal antibodies may display a complex purity/impurity profile that should be assessed by a combination of orthogonal methods, and for which individual and/or collective acceptance criteria should be established for relevant product-related variants. For example, separation methods based on charge heterogeneity should be considered to quantitatively and qualitatively monitor charge variants.

Chromatographic and/or electrophoretic methods capable of detecting product truncation, dissociation and polymerisation should be included, and quantitative limits should be proposed for these, as appropriate.

Particular attention should be paid to the demonstration of the suitability of the analytical methods used to control multimers and aggregates.

Considering that glycosylation may have an impact on the pharmacokinetics of the product, and may modulate its immunogenic properties, appropriate acceptance criteria should be considered for this attribute. In addition, such control will further confirm the consistency of the product.

As a consequence, tests and acceptance limits for relevant glycosylation structures should be carefully considered (e.g. relative amounts of G0, G1 and/or G2 of Fc fragments, levels of galactosylation, fucosylation and sialylation) taking into account the intended and potential impact of this attribute on the biological activity in the context of the clinical situation (e.g. the presence of functional effector functions not being required for the intended mechanism of action, Fab glycosylation).

The control of relevant process-related impurities should be included in the control strategy. In some situations, and where appropriately demonstrated, their control may be performed on an intermediate product, at an appropriate process step. Routine testing may not be necessary for some impurities for which the process has been demonstrated to achieve high reduction levels. Control of residual protein A, HCP, residual DNA and other potential culture or purification residues are typically part of the drug substance specification, as appropriate. In addition, such control provides valuable information on process consistency and performance.

4.4.3. Potency

Potency is the quantitative measure of biological activity based on an attribute of the product which is linked to the relevant biological properties. A relevant potency assay should be part of the specifications for drug substance and/or drug product, and should ideally reflect the biological activity in the clinical situation.

For antibodies for which the clinical activity is only dependent on binding/neutralising properties, a potency assay that measures binding to the target (i.e. binding assay) may be deemed acceptable, if appropriately justified. Where effector functions are relevant for clinical activity, a cell-based bioassay or another assay that takes effector functions into account should be performed. A combination of two separate methods, one measuring the specificity and one giving an indication of an effector function (e.g. complement activation, C1q binding, Fc gamma receptor binding) may be acceptable if a cell-based assay is not feasible or if the combination of two methods gives more precise results.

Although the two types of potency assays (binding or cell-based) often yield comparable results, these assays cannot be deemed interchangeable, because there are product attributes that may not affect binding to target (e.g. glycosylation, fragmentation) but may affect further signalling or receptor expression.

Specific activity (biological activity per mass) is of considerable value to demonstrate consistency of production.

4.4.4. Quantity

The quantity of the drug substance, usually based on protein content (mass), should be determined using an appropriate assay.

4.4.5. General tests

Appearance, solubility, pH, osmolality, extractable volume, sterility, bacterial endotoxins, stabiliser and water, should be assessed where appropriate.

Visible and sub-visible particulate matter in drug product should comply with the requirements set forth in the European Pharmacopoeia.

5. MONOCLONAL ANTIBODY-RELATED PRODUCTS

In addition to intact, non-modified monoclonal antibodies, the scientific principles described in this document can be applied to other monoclonal antibody related products, such as antibody fragments (including single-chain variable fragment (scFv)), fusion proteins, conjugated monoclonal antibodies, bispecific antibodies and radiolabelled antibodies. However, their applicability will be determined on a case-by-case basis, based on the specific properties of the product.

Additional monoclonal antibody-related product specific annexes will progressively be developed and will be made available on the EMEA website, as appropriate.

6. REFERENCES

- ICH Q5A (R1) "*Viral safety Evaluation of Biotechnology Products derived from Cell Lines of Human or Animal Origin*" (CPMP/ICH/295/95)

- ICH Q5B "*Analysis of the Expression Construct in Cell Lines used for Production of r-DNA derived Protein Products*" (CPMP/ICH/139/95)

- ICH Q5D "*Derivation and Characterisation of Cell Substrates used for Production of Biotechnological/Biological Products*" (CPMP/ICH/294/95)

- ICH Q5E "*Comparability of Biotechnological/Biological Products subject to Changes in their Manufacturing Process*" (CPMP/ICH/5721/03)

- ICH Q6B "*Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*" (CPMP/ICH/365/96)

- Note for Guidance on "*Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products*" (EMEA/410/01)

- Guideline on "*Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins*" (CHMP/BMWP/14327/06)

- Guideline on "*Strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products*" (EMEA/CHMP/SWP/28367/2007)

- Ph. Eur. Monograph on "*Monoclonal antibodies for human use*" (2031)

- Ph. Eur. Monograph on "*Human normal Immunoglobulin*" (0338)

- Ph. Eur. Monograph on "*Parenteral preparations*" (0520): *2.9.19. Particulate contamination: sub-visible particles* (20919)

- Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the "*Community code relating to medicinal products for human use*", as amended

ANNEX I - Suggested list of human tissues to be used for immunohistochemical or cytochemical investigations of cross reactivity of monoclonal antibodies.

This list should reflect the specificity of the antibody and its particular use.

- Tonsil, thymus, lymph node;
- Bone marrow, blood cells;
- Lung, liver, kidney, bladder, spleen, stomach including underlying smooth muscle, intestine,
- Pancreas, parotid, thyroid, parathyroid, adrenal, pituitary;
- Brain, peripheral nerve;
- Heart, striated muscle;
- Ovary, testis;
- Skin;
- Blood vessels.