Setting Up a Rapid Mycoplasma Assay to Support Recombinant Protein Production

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ThermoFisher SCIENTIFIC

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Octapharma AB (OAB) in Stockholm, Sweden, is the site for Nuwiq human recombinant factor VIII (FVIII), production. The drug is produced in a human cell line cultured in a perfusion bioreactor using a closed system (to minimize contamination) and proprietary serum-free medium without animal-derived components.

In accordance with regulatory guidelines, cell banks and cell cultures used for production of biological products must be free of mycoplasma. Traditional mycoplasma testing is a growth-based method that represents a significant bottleneck in quality control (QC) testing, and it is expensive and cumbersome. Consequently, many companies outsource this testing, which can add to the turnaround time.

In 2014, the mycoplasma project was established at OAB's plasma quality department. The mycoplasma project team investigated alternatives to growth-based mycoplasma testing for bulk-harvest material. The department performs polymerase chain reaction (PCR) testing of donor plasma on two million samples annually and holds extensive experience and knowledge with PCR method development. To meet those sample processing demands, a new laboratory space was established requiring new equipment and qualified instruments.

The project team evaluated whether to use a commercially available mycoplasma assay or to develop an in-house assay. An in-house assay was determined to be too time consuming, requiring development of several primer sets to cover the mycoplasma species required by regulatory guidelines. In addition, extra time would have been needed to define suitable instrumentation and compensate for the lack of background within the regulatory field concerning mycoplasma. At the time, several pharmaceutical companies were

About Octapharma AB

Founded in 1983 by Wolfgang Marguerre and headquartered in Lachen, Switzerland, Octapharma is one of the largest human-protein manufacturers in the world. About 8,000 employees worldwide are active in three therapeutic areas: hematology, clinical care, and immunotherapy. The company serves patients in 113 countries through offices in 32 countries. Its five European production sites are located in France, Germany, Sweden, and Switzerland.

The Octapharma Stockholm site (OAB) was established in 2002. This site has more than 800 employees and produces several of Octapharma's plasma-derived products (e.g., Octagam, Octanate, Octaplas, Albunorm, Gammanorm, and Rhesonative) as well as recombinant-protein biomanufacturing of Nuwiq recombinant human factor VIII.

Figure 1: MycoSEQ analysis

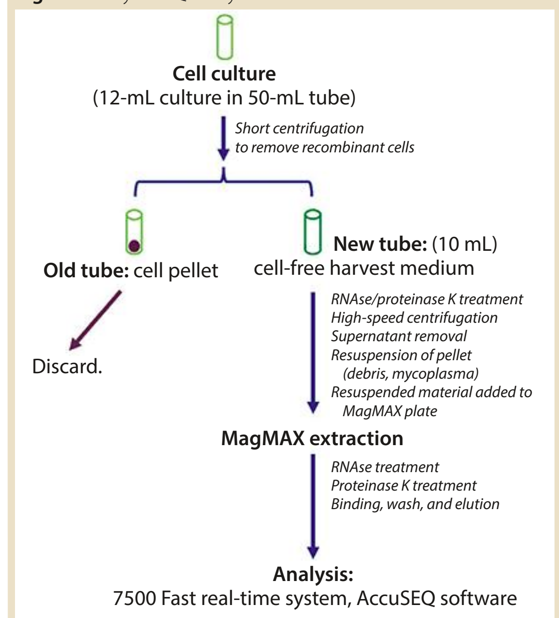
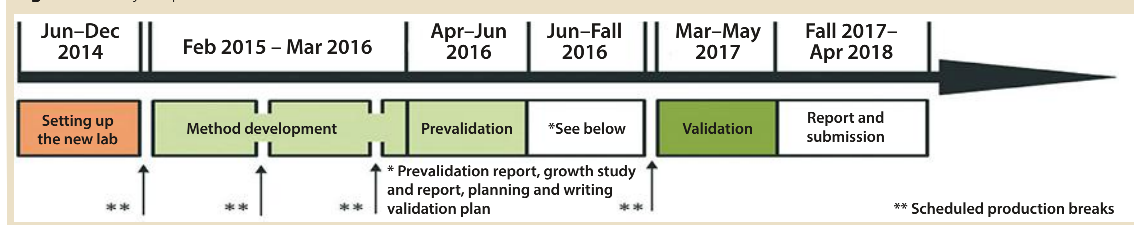


Figure 2: Project phase timeline



already in the regulatory approval process with Thermo Fisher's MycoSEQ assay. In addition, Thermo Fisher Scientific offered comprehensive support by including training, instrument qualification services, assay-optimization support, validation study design, and support with regulatory submissions. For these reasons, Thermo Fisher Scientific's MycoSEQ mycoplasma detection assay was the most suitable option.

The MycoSEQ Mycoplasma Detection System

The MycoSEQ method is a SYBRGreen-based qPCR analysis that can detect >90 different mycoplasma species. The assay uses a discriminatory positive control (DPC): a synthetic DNA molecule mimicking mycoplasma as positive, internal, and external controls.

The Assay Approach: The default approach for analysis is a direct extraction of fresh 10-mL bulk harvest material. However, some material may not be optimal for the direct method because of very high cell densities, large amounts of cellular debris, and/or certain media components present. In such cases, an enrichment procedure to promote mycoplasma growth may be necessary before analysis. Preliminary studies concluded that the direct method was suitable for OAB bulk harvest material.

Key Instruments and Software: Samples were processed using Thermo Fisher Scientific's PrepSEQ 1-2-3 nucleic-acid extraction kit. Hands-on time was reduced by using a MagMAX *Express*-96 deep-well magnetic particle processor. An Applied Biosystems (AB) 7500 Fast real-time PCR system and AccuSEQ 2.0 real-time PCR analytical software from Thermo Fisher Scientific were used for PCR and subsequent data analysis. The AccuSEQ software package was developed for quantitative PCR (qPCR) testing of impurities and contaminants in biopharmaceutical manufacturing processes, and it offers security, auditing, and electronic-signature capabilities to meet 21 CFR Part 11 compliance. The software also allows for automated mycoplasma detection.

The Assay: Analysis starts with 12 mL of fresh bulk harvest. The sample is centrifuged to remove the recombinant cells, which are discarded. Then 10 mL of cell-free bulk harvest supernatant is subjected to enzymatic digestion and centrifuged at high speed to pellet what mycoplasma may be present. The supernatant is removed, and the pelleted material is resuspended for MagMAX extraction. Mixed with MycoSEQ primers and a buffer, the MagMAX eluate is analyzed by the 7500 Fast real-time instrument and AccuSEQ software (Figure 1).

Project Phases in Detail

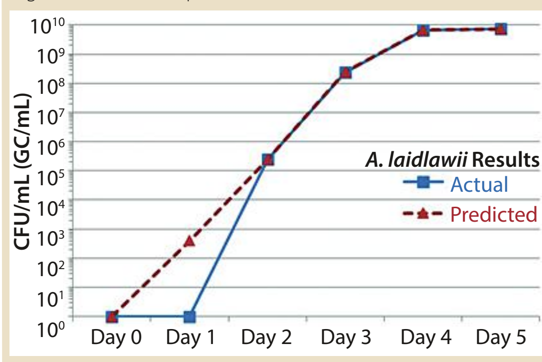
Implementation of this project took about four years from the point of investigating the PCR method as an alternative to the traditional method to regulatory submission (Figure 2). Thermo Fisher Scientific provided technical support and assistance throughout all phases of the project.

Laboratory Set-Up: The project required establishment of a new laboratory. This included purchasing and qualifying new equipment/instruments, initiating establishment of standard operation procedures (SOPs), and MycoSEQ assay training. Equipment that required qualification included a microbiological safety hood, a high-speed centrifuge, the MagMAX instrument, the PCR workstation, and the real-time PCR instrument and software. The laboratory space was designed to segregate steps of the workflow.

Method Development: Preliminary studies had demonstrated that the direct method was suitable for the bulk harvest matrix; however, assay development and optimization were necessary to ensure sensitivity to 10 GC (CFU)/mL as required by regulatory guidance. Recombinant cell-removal conditions, MagMAX enzymatic digestion, extraction wash, elution volume, and PCR annealing temperatures all were optimized.

Prevalidation: Two bulk harvests were spiked with genomic DNA (gDNA) from six mycoplasma species at 10 GC/mL and analyzed. Additionally, one viable mycoplasma was spiked at the limit of detection (LoD) of 10 CFU/mL. Problems with

Figure 3: Analyzed growth data for *Acholeplasma laidlawii*, Days 0–5; the predicted data for Day 1 (red) was missed probably because of the smaller extraction volume. Rapid growth to very high titers made it necessary to use a *y*-axis log scale for all data points to be included.



mycoplasma aggregation were resolved so that excellent results ultimately were obtained.

Supporting Study: A supporting growth study was performed to mimic a low-level mycoplasma contamination in our bioreactors and to determine how soon such an infection could be detected by MycoSEQ analysis. Mycoplasma growth was performed by an Austrian laboratory that specializes in mycoplasma and has dedicated space for growth studies. The timing and logistics of shipping live cells was difficult, so cell-free bulk-harvest material rich in media nutrients, cell metabolites, and cell debris was substituted. Samples were harvested for six continuous days at the Austrian site, then lysed, frozen, and sent to OAB for analysis.

Compiled from growth-study data, Figure 3 illustrates the rapid growth of *Acholeplasma laidlawii* mycoplasma. If fresh samples had been processed, however, with debris removal followed by analysis or using a 10-mL direct method for bulk harvest, then *A. laidlawii* easily could have been detected at 24 hours post infection (indicated by the orange line). At the 36-hour time point, growth had passed 1×10^5 CFU/mL.

Validation: After a scheduled winter production break in 2017, validation began and lasted three months, with a report written in the fall of 2017 and submitted in April 2018. Two factors made the validation process more straightforward than it might have been otherwise: the large number of MycoSEQ users with regulatory approval worldwide already and complementary data provided by Thermo Fisher Scientific.

For validation, gDNA from only two species, *A. laidlawii* and *Mycoplasma arginini*, was used. They represent the range of results for all six species used in prevalidation and represent species that could be of highest threats theoretically for OAB's Stockholm site. Mycoplasma gDNA was spiked at LoD, with 24 PCR replicates of each species. Validation focused on robustness to address bulk-harvest variation. Stability of our bulk harvest and extraction eluate and varying intermediate precision of analytical personnel also were evaluated.

Results and Submission: Initial plans were to use nine different bulk harvests; however, material from 11 harvests was used with 18 separate extractions and 21 independent qPCR analyses. Excellent validation results met the 10-GC/mL (10-CFU/mL) regulatory requirement. The validation report was written and reviewed, and the validation package was compiled (consisting of a validation report, an appendix with an executive summary on the prevalidation, and the prevalidation report) and submitted to Octapharma's regulatory group in Vienna. Material was submitted to the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and Health Canada in April 2018.

Recommendations

When working with the MycoSEQ method, manual pipetting of nearly full 96-well plates can be difficult, especially when performing several assay modifications or optimizations. Implementation of a pipetting robot or 96-well pipetting guide is beneficial during validation, when plates can be complex. This type of information helps save reagents and prevent manual errors.

Because of the scale of this project, early communication with all relevant departments was critical to success. The test method requires optimization for a given matrix. It is critical to understand the process and be aware of material availability for assay development and validation work. Finally, include as many different harvests as possible within process specification limits so that different cell densities and viabilities can be tested.

Kent Persson, *PhD, is project manager for mycoplasma work at Octapharma AB in Stockholm Sweden.* *Nuwiq, Octagam, Octanate, Octaplas, Albunorm, Gammanorm, Rhesonative, MycoSEQ, PrepSEQ, MagMAX, AccuSEQ, and SYBRGreen are registered trademarks of their respective owners.*