Application Note

Rapid Detection of Slow-growing Anaerobe Using MiQLab™ System

Eric C. DiBiasio, Manoj Nair, Zihua Wang, Diane Migneault, and Nathan Walsh

- At-line rapid PCR can detect slow-growing anaerobe and typical bioreactor contaminant, *Cutibacterium acnes* (*C. acnes*), at least 36-times faster than conventional methods.
- Rapid screening system delivers accurate and reliable results with a 2 hr turnaround time.
- MiQLab decreases time to results with less than 1 min hands-on time and fully automated analysis workflow.

Introduction

Mammalian cell culture represents a critical aspect in the biopharmaceutical manufacturing of monoclonal antibodies, viral vaccines, and other therapeutic proteins. Routine microbial contaminant monitoring of in-process cell culture samples is essential to maintain quality, efficacy, and safety of a product during manufacturing. Failure to do so can cost a company hundreds of thousands of dollars to as high as millions of dollars. A major factor that can dramatically influence effective microbial contaminant detection and ultimate response is the turnaround time (TAT) associated with proper identification.[1]

Conventional cell culture is used for microbial contaminant detection and is implemented using membrane filtration, with subsequent transfer to high nutrient agar incubated to enrich for growth. This method is sufficient to test for fast growing bacterial contaminants but is inadequate for a slow-growing bacterium. Typically, the most common microbial cell culture contaminants are human skin bacteria (i.e., *Cutibacterium* spp. and *Staphylococcus* spp.) and spore-forming bacteria (i.e., *Bacillus* spp.), with the most rare being gram-negative bacteria.[2, 3] *Cutibacterium* spp. account for nearly 50% of a human's skin microbiota where it is primarily associated with hair follicles.[4-6] In the case of *Cutibacterium*, the main species of interest is *Cutibacterium (Propionibacterium) acnes* (*C. acnes*), a slow-growing, aerotolerant anaerobic, gram-positive bacterium linked to contributing to human acne.[7] *C. acnes* is found with the highest frequency on the face, scalp, upper chest, shoulders, and back.[4-6, 8–12] Further, *C. acnes* can be spread through dead cells or skin flake shedding.[13] For this reason, there is a constant human operator mediated mammalian cell culture bioreactor contamination risk and thus it is not surprising that *Cutibacterium* spp. are common contaminants.[14]

Critically, more rapid detection methods are needed for cell culture microbial contaminants, and LexaGene's MiQLab System can provide that capability. MiQLab is a fully automated, rapid, sample-to-answer molecular diagnostic system that has been engineered to detect pathogens, or in this case, microbial contaminants. MiQLab uses the gold standard molecular diagnostic technique of real-time or quantitative Polymerase Chain Reaction (qPCR), which has high sensitivity, specificity, and broad multiplexing capabilities desirable for specific microbial detection at the point-of-need.

The open-access capability and flexibility of the MiQLab gives biopharmaceutical manufacturing companies the ability to perform rapid, at-line, microbial contamination testing with ~2 hr TAT to results. To assess the performance of the MiQLab System at detecting a slow-growing bacterium associated with bioreactor contamination, which is a major bottleneck and challenge in the manufacturing process, we performed a competition study.

Methodology

In this study, slow-growing bacterium and typical contaminant, *C. acnes*, was challenged for microbial detection utilizing both LexaGene's MiQLab System and anaerobic culture. The designed study began by growing a pure culture of *C. acnes* in a rich liquid medium until turbid and determining its titer which was found to be 1 x 10^9 colony forming units per milliliter (CFU/mL). Subsequently, a 5-log dilution was performed into fresh culture medium representing a nominal pull from a contaminated bioreactor, signifying 0 hr. Cultures were incubated at 37°C and grown for an additional day, signifying 24 hr (Figure 1). At both 0 hr and 24 hr, 1 mL of culture was aliquoted into a MiQLab Sample Prep Cartridge and successively ran on the MiQLab System, while further dilutions were made to enumerate bacterial counts.

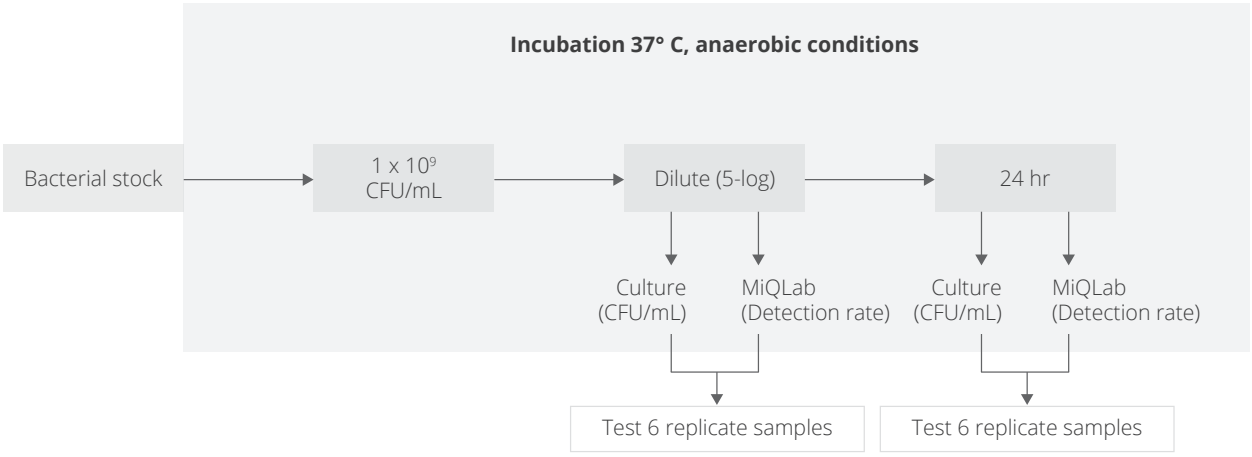

Figure 1. Workflow for *C. acnes* study.

Results and Discussion

The MiQLab was able to rapidly detect *C. acnes* at both 0 hr and 24 hr with a ~2 hr TAT, demonstrating a 100% detection rate (6 out of 6 detected) at 24 hr with a mean cycle threshold (Ct) value of 20.7 (Table 1). In contrast, a pure culture (with concentration expressed as CFU/mL) took an extended 3-day period for detection (Table 2).

Table 1. MiQLab performance results.

Time	Turnaround time	Total valid results	Hit rate	Mean Ct
0 hr	**~2 hours**	12	50%	25.0
24 hr			100%	20.7

Table 2. *C. acnes* culture results.

Time	Turnaround time	Concentration (CPU/mL)
0 hr	**3 days**	10,000
24 hr		1,000,000

C. acnes, as previously described, is a slow-growing anaerobic bacterium, "where if bioreactor contamination is suspected, detection can be achieved by anaerobic culture where primary growth (containing a mixed population) can take up to 14 days, not including the additional time needed for subculture and confirmatory testing."[15] Correct identification requires skilled technical interpretation and laborious manual steps. For example, expertise is required to aseptically culture a microbial contaminant, perform the necessary subculturing steps, and conduct microscopic observations for the presence of microorganisms.[14] All these tasks increase the time to results, in addition to the lengthy incubation period.

Reducing the time to results for detecting microbial contaminants can have a substantial influence on the biological therapeutic product being produced. Testing the raw materials, seed cultures, scaled up material, and the final product, are all imperative to ensure stability and conformity. Contamination can ultimately decrease potency through enzymatic modification or degradation of the product, elevated levels of bacterial endotoxins, and alterations in impurity profiles,[16, 17] all causes of product variability. Trying to identify the cause of a microbial contamination is a hindrance in manufacturing operations and can result in prolonged shutdown periods. Collectively these issues contribute toward a significant loss to the biopharmaceutical manufacturing company.

Conclusions

In this study, LexaGene's MiQLab System has demonstrated the ability to dramatically decrease the time to results and deliver high sensitivity results within two hours. The MiQLab can perform as much as 168-times faster, if implemented as an at-line bioreactor microbial contaminant detection system, compared to traditional culture (2 hr vs 14 days). Implementing the MiQLab for microbial contamination identification of slow growing anaerobes such as *C. acnes* can eliminate the incubation time bottleneck associated with slow growing bacterial contaminants, potentially leading towards substantial cost savings for biopharmaceutical manufacturers.

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500 Cummings Center, Suite 4550
Beverly, MA 01915 USA
LexaGene.com

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