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Strategy for an abbreviated in-house qualification of a commercially available Rapid Microbiology Method (RMM) for canadian regulatory approval

JOLENE CHISHOLM[1,2], SHASHANK BHATT[1,2], AMÉLIE CHABOUREAU[1,2] & SOWMYA VISWANATHAN[1,2,3]

[1]The Arthritis Program, Krembil Research Institute, University Health Network, Toronto, Canada, [2]Cell Therapy Program, University Health Network, Toronto, Canada, and [3]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada

Abstract

Background aims. Cell therapy products (CTP) typically require full sterility, endotoxin and *Mycoplasma* testing before product release. Often this is not feasible with fresh cells, and sponsors may rely on rapid microbiological methods (RMM). RMM must be qualified in-house using the sponsor's facilities, equipment, consumables, cells and matrices to meet regulatory approval. Herein, we present a cost-effective strategy to conduct an in-house abbreviated qualification of a commercially available RMM kit to meet Health Canada regulatory requirements. *Methods.* We performed an abbreviated qualification using a polymerase chain reaction (PCR)-based *Mycoplasma* testing method involving assay sensitivity and ruggedness, based on an experimental plan that was pre-approved by Health Canada. Briefly, investigational CTPs were tested in-house using a PCR-based *Mycoplasma* detection kit. Assay sensitivity was determined using a 10-fold dilution series of genomic DNA of only two *Mycoplasma* species, *Mycoplasma arginini* and *Mycoplasma hominis* in the absence of CTP-matrix as the kit had been previously validated against nine species. Matrix interference was measured by testing independent CTP samples. Testing by different operators on different days measured ruggedness. *Results.* The RMM *Mycoplasma* qualification exceeded sensitivity (4 genome copies per reaction for *M. arginini* and 0.12 genome copies per reaction for *M. hominis*) and met ruggedness requirements without matrix interference, as required by the Pharmacopoeial guidelines (Ph. Eur. 2.6.7 and USP <1223>). *Discussion.* Our approach represents a minimal qualification that can be performed by an academic institution while ensuring regulatory compliance for implementing RMM testing for in-process and product-release testing of CTPs.

Key Words: *cell therapy products (CTPs), mycoplasmas, nucleic acid amplification technique (NAT), rapid microbiological method (RMM), qualification, pharmacopeia*

Introduction

The increased development interest and commercialization of cell-based therapeutics is expected to reach $145.8 billion USD on the global market by 2021 [1]. This has accelerated interest in rapid microbiological methods (RMM) because most cell therapy products (CTPs) cannot undergo terminal sterile filtration or sterilization and have a short shelf life, which often necessitates administration to a patient before sterility or *Mycoplasma* test results from traditional culture methods are obtained. RMM technologies can identify microbiological risks faster than traditional culture methods, reduce product release time and contribute to continual improvement of manufacturing processes through the generation of high throughput, near real-time data, all of which enhances the safety profile of CTPs while decreasing overall cost and labor. In 2004, the U.S. Food and Drug Administration (FDA) approved a sterility RMM for lot-release of a CTP manufactured by Genzyme, Carticel, 7 years after initial approval and after extensive qualification [2].

Although compendium methods that involve the isolation and/or culture of microorganisms have been traditionally accepted, regulators have encouraged the use of RMM as an alternative. In fact, the General Biological Products Standards section of the Code of Federal Regulations in the USA (21 CFR Part 610.9) describes equivalent methods and processes

Correspondence: **Sowmya Viswanathan**, PhD, The Arthritis Program, Krembil Research Institute, University Health Network, 60 Leonard Avenue, Room 3 KD479, Toronto, Ontario, Canada, M5T 2S8. E-mail: sowmya.viswanathan@uhnresearch.ca

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for testing methods [3]. The U.S. Pharmacopeia (USP) and European Pharmacopeia (Ph. Eur.) have relevant chapters describing qualification of alternative microbiological assays: USP <1223 > "Qualification of Alternative Microbiological Methods" [4], Ph. Eur. 5.1.6. "Alternative Methods for Control of Microbiological Quality" [5] and Ph. Eur. 2.6.27 "Microbiological Control of Cellular Products" [6]. Moreover, publications by scientists at regulatory agencies provide further evidence of support for RMM assays [7–9].

Mycoplasmas are common contaminants in continuous cell cultures in research laboratories [10–12]. Routes of *Mycoplasma* contamination in CTP manufacturing facilities include materials, reagents (e.g., fetal bovine serum [FBS]), personnel and donor tissues. Although manufacturing in a Good Manufacturing Practice (GMP) environment seeks to control and eliminate these sources of *Mycoplasma* contamination, lot-release testing confirming the absence of *Mycoplasma* contamination is still a regulatory requirement to ensure product safety before infusion into patients [13–15].

RMM, such as nucleic acid amplification technique (NAT), are accepted by regulators as an alternative detection system to culture and indicator methods for detection of *Mycoplasma* [3,16]. However, RMM must be qualified in-house using the sponsor's facilities, personnel, equipment, consumables, cells and matrices. Ph. Eur. 2.6.7 specifically outlines *Mycoplasma* NAT qualification requirements [17], and USP <1223 > and Parenteral Drug Association Technical Report #33 provide general guidance on validating alternative microbial methods [4,18]. According to these guidelines, the alternative test must yield results that are equivalent or better than the compendium method specifically in areas of accuracy, specificity, sensitivity, robustness and ruggedness.

We qualified a commercially available PCR-based *Mycoplasma* detection assay using an abbreviated qualification plan which was pre-approved by Health Canada for detection of *Mycoplasma*s in an investigational CTP—autologous mesenchymal stromal cells (MSCs) for treating patients with knee osteoarthritis (Health Canada parental control #177258). We established an abbreviated qualification concept based on Ph. Eur. 2.6.7 and USP <1223 > , limited to sensitivity, detection limit and ruggedness [19]. We leveraged previous validation documented by the kit manufacturer [20] and an internal risk analysis underlining the absence of *Mycoplasma* contamination in our GMP cell manufacturing facility. Our abbreviated qualification plan represents a cost-effective strategy for academic health care centers undertaking clinical investigations with limited resources and can be applied to other RMMs such as rapid sterility and/or microbial identification methods.

Methods

Positive control (mycoplasma) samples

Genomic DNA of *Mycoplasma arginini* strain G230 (ATCC qCRM-23838D) and *Mycoplasma hominis* (ATCC qCRM-27545D) were spiked into matrix medium to serve as positive control samples. These strains are certified reference material produced under ISO Guide 34:2009 at ISO/IEC 17025:2005 and ISO 9001:2008 accredited/certified laboratories.

Test samples

MSC products were prepared according to previous clinical studies [21] and were harvested in-process (7–14 days before final harvest), and on the day of the scheduled MSC injection (final product). The test samples were composed of $2–3 \times 10^6$ patient-specific MSCs in 450 µL of cell culture medium collected before cell washing and final product formulation to maximize the possibility of detecting a *Mycoplasma*-contaminated sample, as discussed with Canadian regulators. All patients were consented under UHN REB #14-7909-B, and the study was approved by Health Canada (parental control #177258; clinicaltrials.gov identifier NCT02351011).

PCR primers

Universal 16s rRNA Mycoplasma primers validated by the kit manufacturer for the detection of *M. arginini, M. pneumoniae, M. fermentans, M. hyorhinis, M. orale, M. salivarium, M. hominis, Acholeplasma laidlawii* and *Spiroplasma citri* provided in the MycoTOOL PCR Mycoplasma Detection kit (Roche Diagnostics) were used [20]. These primers have limited cross-reactivity with phylogenetically similar Gram-positive species: *Streptococcus bovis* (non-reactive up to 10^6 colony-forming units [CFU]/mL), *Lactobacillus acidophilus* (cross-reactive at $\geq 10^3$ CFU/mL) and *Clostridium sporogenes* (cross-reactive at $\geq 10^3$ CFU/mL) [20].

In addition, as a DNA extraction control, primers targeting human β2-microglobulin gene (B2M) were synthesized (Invitrogen): forward primer (sequence: 5′-ACT CTG GGT TTT CGT GAC TCT -3′); reverse primer (sequence: 5′-TTT GGA GTA CGC TGG ATA GCC T-3′). The B2M primers were used in lieu of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers provided with the kit, which were designed for use with Chinese hamster ovary (CHO) cells and were unable to detect the human GAPDH gene.