ATCC

APPLICATION NOTE

DEVELOPMENT AND PERFORMANCE EVALUATION OF A MODIFIED ABP-FREE MCCOY'S 5A MEDIUM FORMULATION: MCCOY'S 5A MEDIUM, ABP-FREE (ATCC® 30-2011™)

Tiffany Cato, MS; Nancy Palechor-Ceron, MS, DMD
ATCC, Manassas, VA 20110

ABSTRACT

Recent concerns over the introduction of adventitious pathogens through animal by-product derived components in cell culture media have led researchers to prefer animal by-product (ABP)-free media for cell culture. ATCC has developed a modified ABP-free McCoy's 5A medium formulation: McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™). The newly developed medium was tested across several ATCC cell lines for performance and was found to be a suitable substitute for the current McCoy's 5A Medium (ATCC® 30-2007™) formulation with no significant impact to cell health or cell proliferation.

INTRODUCTION

Cell culture plays a critical role in advancing human understanding of biology, medicine, and biotechnology. Cell culture media, which provide the necessary nutrients and environment for cells to thrive, are a vital component of these experiments. Increasingly, animal by-products (ABPs) in cell culture media have become a point of consideration when selecting reagents for research. The use of ABP-free reagents improves experimental reproducibility, reduces contamination risk, and aligns with evolving regulatory and safety requirements. To support research and industry needs, ATCC has reformulated McCoy's 5A Medium (ATCC® 30-2007™) to remove ABPs.

McCoy's 5A medium was originally developed in 1958 by Neuman and McCoy to culture Walker 256 cells and Novikoff hepatoma cells in vitro.[1] Neuman and McCoy modified the formulation of Eagle's Minimum Essential Medium (EMEM) and added 1% yeast extract to evaluate the effects of various amino acids on Novikoff hepatoma cells in vitro. They found that L-asparagine and glutamine, as well as 12 other amino acids, were essential for the continuous culture of Novikoff hepatoma cells. This formulation was later modified by Iwakata and Grace for the culture of acute myeloblastic leukemia cells.[2] Iwakata's modification included 0.06% Bacto™ Peptone as well as increased amounts of folic acid and vitamin B12.[3] Iwakata's modifications were widely adopted and are the basis for the McCoy's 5A Medium (ATCC® 30-2007™) formulation at ATCC.

In the formulation, Bacto™ Peptone acts as an additional nitrogen source in the culture medium.[4] This commercially available supplement is composed of an enzymatic digest of bovine and porcine animal proteins. Therefore, to develop a ABP-free alternative to ATCC McCoy's 5A Medium (ATCC® 30-2007™), we removed this animal-based peptone from the formulation to create McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™). In this study, we evaluated the performance of the ABP-free formulation to ensure its suitability as a substitute for McCoy's 5A Medium.

MATERIALS AND METHODS

CELL LINES:

In this study, we evaluated the performance of McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) using the following cell lines: HCT 116 (ATCC® CCL-247™), MES-OV (ATCC® CRL-3272™), HT-29 (ATCC® HTB-38™), SK-ES-1 (ATCC® HTB-86™), and Kasumi-3 (ATCC® CRL-2725™). With the exception of the Kasumi-3 cell line, the cell lines selected utilize the current ATCC McCoy's 5A medium as their basal medium. Kasumi-3 was selected as this cell line shares multiple similarities to the cell line used by Iwakata during the development of the modified McCoy's 5A formulation with peptone; both cell lines are lymphoblast cell lines that were isolated from the peripheral blood of a patient with acute myeloblastic leukemia (AML).

PREPARATION OF COMPLETE CELL CULTURE MEDIA FOR TESTING:

Complete culture media for each cell line was prepared in accordance with ATCC cell line specifications. Control medium was prepared using McCoy's 5A Medium (ATCC® 30-2007™) as the basal media. Test medium was prepared using McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) as the basal media. All preparations used the same lot of fetal bovine serum (FBS; ATCC® 30-2020™) to control for variations in sera lots. Cells were thawed directly into complete culture media containing McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) without an adaption period for all experiments.

MEDIA PERFORMANCE EVALUATION:

We evaluated the short-term performance of McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) by measuring cell proliferation during a 48-to-96-hour time period. The MES-OV, HCT 116, and HT-29 cell lines were selected for this performance evaluation due to the cell lines' ability to proliferate at comparable rates to one another and ability to form uniform cell monolayers. Cell proliferation was measured using the xCELLigence® RTCA system (Agilent®). The xCELLigence® RTCA system utilizes electrical impedance as a tool to evaluate real-time proliferation, cell number, size, and shape. This impedance is reported by the instrument software as cell index.

Additionally, we performed a cell viability assay using the CellTiter-Glo® assay (Promega). The purpose of this assay was to measure ATP levels as a means of determining viability and to measure potential cytotoxic effects of the new media formulation. The CellTiter-Glo® assay is a single reagent assay that uses luciferase to quantify ATP. The resulting luminescence values are proportional to the percentage of metabolically active viable cells present. Cells were thawed directly into complete cell culture media prepared with McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) without an adaption period alongside control cell culture media. Cells were seeded in such a way to reach confluence within 48-96 hours, and each experiment was performed in triplicate. We analyzed the data using RTCA Software Pro Version 2.3.2 and Graph Pad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). We determined data significance using 2-way ANOVA with Bonferroni test. We analyzed cell index and relative luminescence units (RLU) (Figure 1) of each media condition. The results of the short-term experiment showed that McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) did not exert cytotoxic effects on any of the cell lines tested and performed equivalently to McCoy's 5A Medium (ATCC® 30-2007™).

We then evaluated the long-term effects of the McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) on the growth rate, morphology, and viability of all 5 cell lines. Here, we propagated the cell lines over a 21-day period of continuous culture. Cell cultures were maintained according to established procedures at ATCC. Cells were thawed directly into complete cell culture media prepared with McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) without an adaption period alongside control cell culture media. Cells were maintained in log phase growth throughout the evaluation through regular subculturing once cultures reached optimal confluence. Population doubling level (PDL), viability, and morphology were recorded at each subculture. PDL refers to the total number of times the cells in the population have doubled since the previous subculture. PDL was calculated with the following formula: $n = 3.32 (\log UCY - \log I) + X$, where n = the final PDL number at end of a given subculture, UCY = the cell yield at that point, I = the cell number used as inoculum to begin that subculture, and X = the doubling level of the inoculum used to initiate the subculture being quantitated.[5] We performed statistical analysis of RLU, cell index, average doubling time, and average viability using 2-way ANOVA with Bonferroni test (Figure 2). Statistical analysis was performed using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

RESULTS

We assessed the short-term proliferation of the HCT 116, MES-OV, and HT-29 cell lines using cell index. We compared mean cell indexes of cells grown in McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) against those cell indexes of cells grown in McCoy's 5A Medium (ATCC® 30-2007™). Our data demonstrate that McCoy's 5A Medium, ABP-Free supports cell growth and proliferation in a manner that was equivalent to the current McCoy's 5A Medium formulation (Figure 1A). We then used the CellTiter-Glo® assay data further to analyze and compare overall cell health and found that cells grown in McCoy's 5A Medium, ABP-Free performed equivalently to cells grown in McCoy's 5A Medium (Figure 1B).

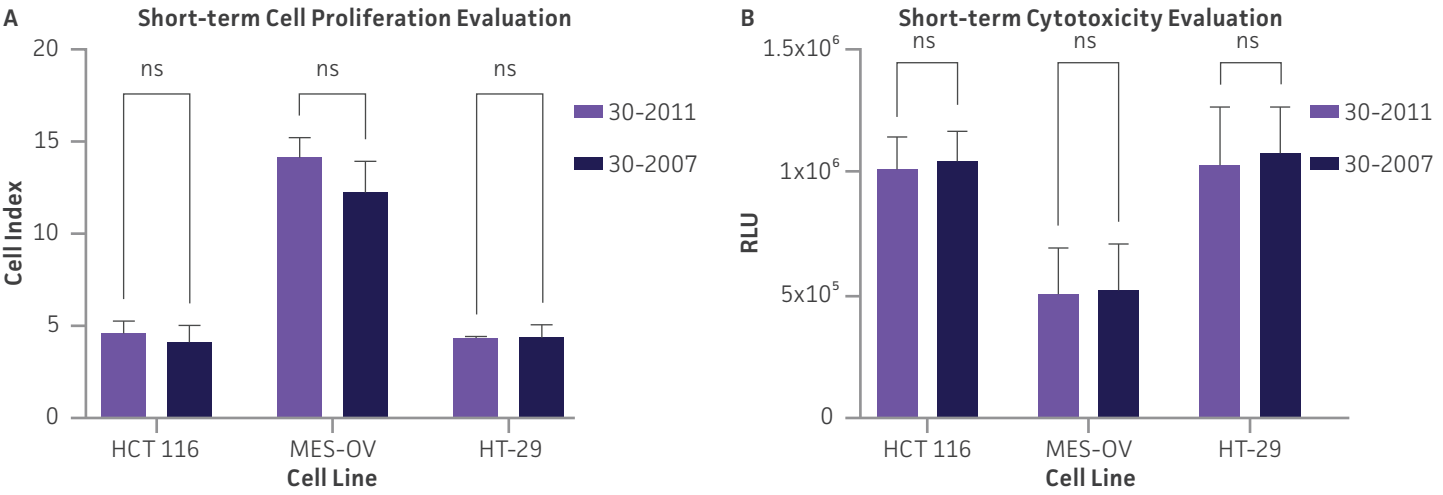

Figure 1: Comparative short-term performance of McCoy's 5A Medium, ABP-Free against McCoy's 5A Medium. (A) Mean cell index comparisons of cell lines grown in McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) and McCoy's 5A Medium (ATCC® 30-2007™). (B) Mean RLU comparisons of McCoy's 5A Medium, ABP-Free and McCoy's 5A Medium. ns (Not Significant) indicates a P value > 0.05.

We then assessed the long-term proliferation results of the five cell lines tested using average doubling time (Figure 2A), average viability (Figure 2B), and population doubling levels (Figure 3) over the course of approximately 21 days. Doubling times and viability were recorded at each subculture and the resulting valued were averaged. We compared mean doubling times and viability of cells grown in McCoy's 5A Medium, ABP-Free against those of cells grown in McCoy's 5A Medium to evaluate cell growth and health during expansion. Our data demonstrate that McCoy's 5A Medium, ABP-Free supports healthy cell expansion similarly to the current McCoy's 5A Medium formulation. We found that the Kasumi-3 cell line grew slightly faster in McCoy's 5A Medium, ABP-Free indicating a slight performance improvement.

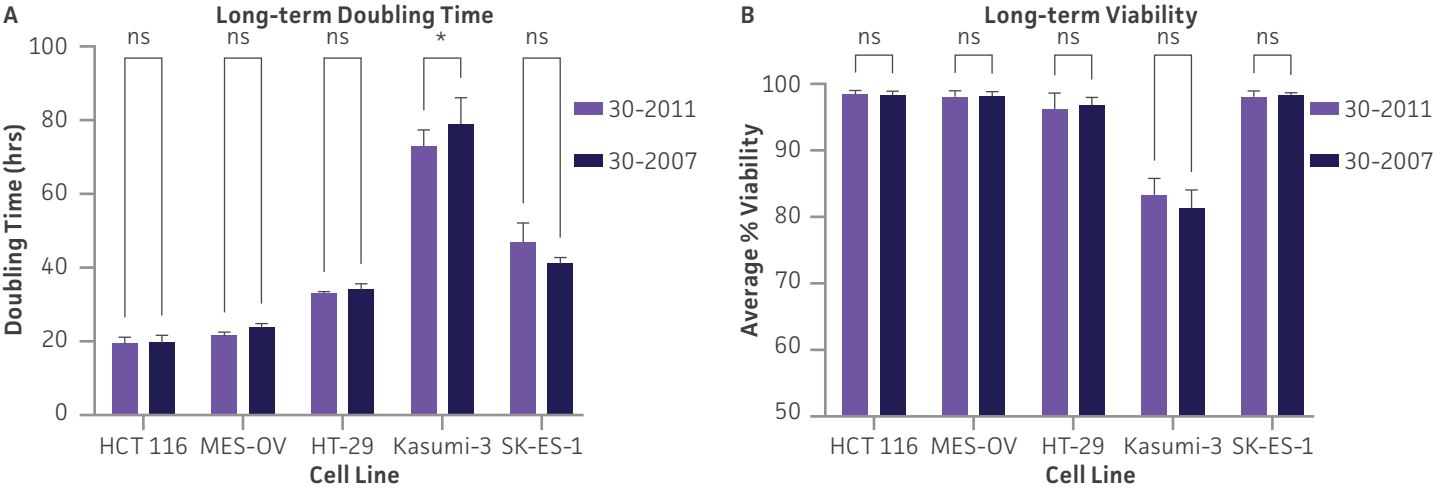

Figure 2: Comparative long-term performance of McCoy's 5A Medium, ABP-Free against McCoy's 5A Medium. (A) Average doubling times of cell lines grown in McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) as compared to those of cells grown in McCoy's 5A (ATCC® 30-2007™). Doubling time was calculated at each subculture and averaged over 21 days in continuous culture. (B) Average percent viability of cell lines grown in McCoy's 5A Medium, ABP-Free as compared to those grown in McCoy's 5A Medium. Viability was measured via the automated trypan blue exclusion method . ns (Not Significant) indicates a P value > 0.05. * indicates P ≤ 0.05

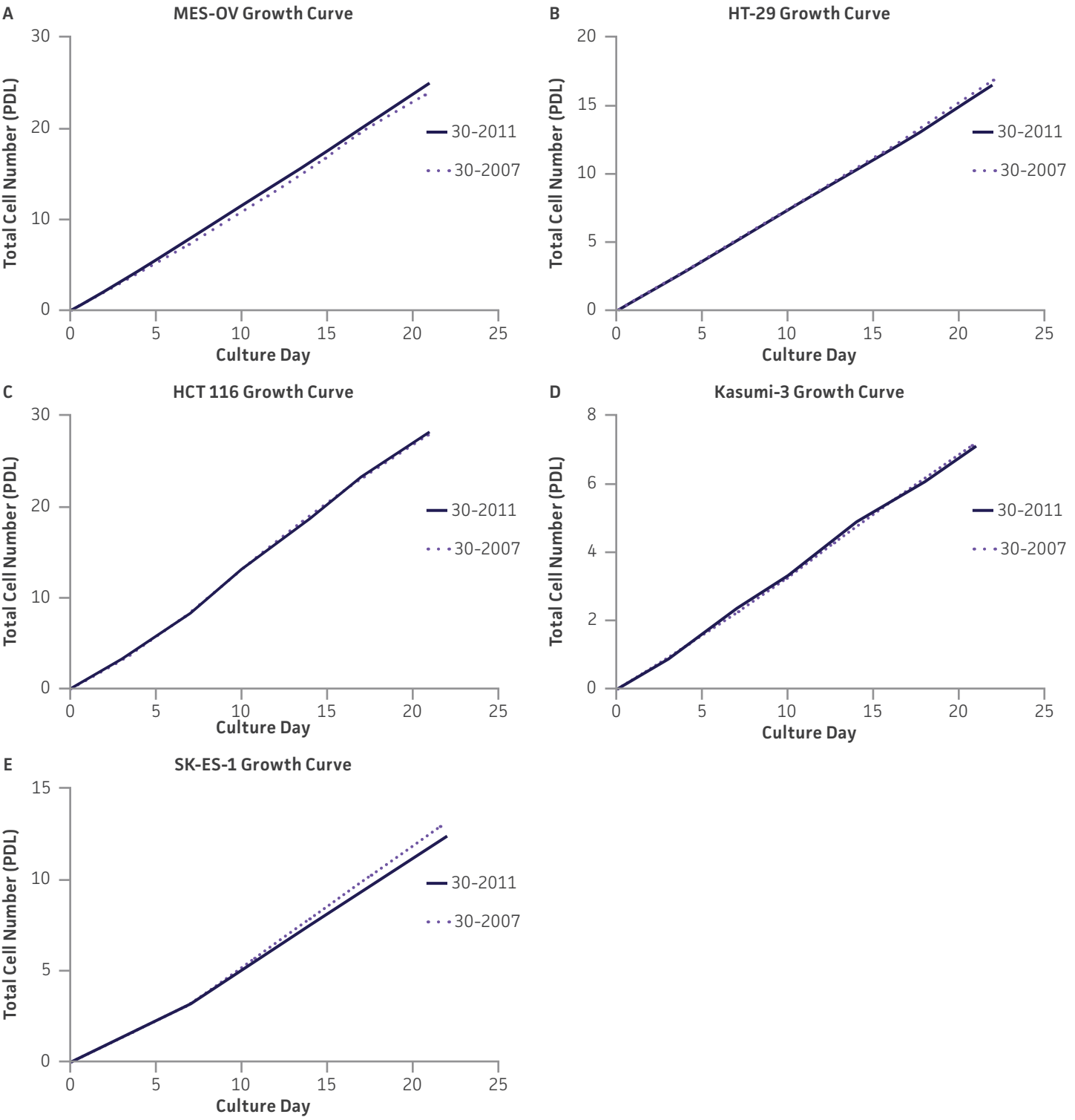

Figure 3: Growth curve comparison of McCoy's 5A Medium, ABP-Free against McCoy's 5A Medium. (A-E) Comparison growth curves showing the performance of cell lines cultured in McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) versus McCoy's 5A Medium (ATCC® 30-2007™). Cell growth is shown as cumulative population doubling levels (PDL) at each subculture.

CONCLUSION

Our data strongly indicate that the McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™) is a suitable ABP-free alternative to the original McCoy's 5A Medium (ATCC® 30-2007™). This ABP-free medium not only successfully supports the long-term growth of cell lines currently cultured in the original McCoy's 5A medium but also exerts no cytotoxic effects on any cell line tested and supports long-term growth of cells without the addition of Bacto™ Peptone. HCT 116, MES-OV, HT-29, and SK-ES-1 cell lines grown in McCoy's 5A Medium, ABP-Free performed equivalently to those grown in McCoy's 5A Medium; the Kasumi-3 cell line performed better when grown in McCoy's 5A Medium, ABP-Free. Furthermore, the data demonstrate that cells may be thawed directly into McCoy's 5A Medium, ABP-Free without the need for an adaption period. Based on this study, ATCC is remanufacturing its McCoy media cell lines in the ABP-free version to better support the scientific community in navigating the safety and regulatory challenges associated with animal by-product-containing cell lines.

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10801 University Boulevard
Manassas, Virginia 20110-2209

703.365.2700 703.365.2701 sales@atcc.org www.atcc.org

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