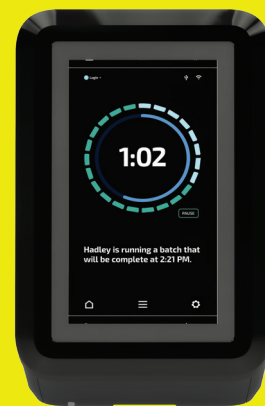


Supplementing RPMI media with different amounts of fetal bovine serum.



BACKGROUND

Roswell Park Memorial Institute (RPMI) developed RPMI-1640 media from modification to McCoy's 5A media. RPMI media is one of the most used chemically-defined and protein-free cell media since it works across a variety of mammalian cell lines (e.g., stem cells, T and B cells, hybridomas, HEK293, THP-1 leukemia cells, etc.) grown in suspensions and as monolayer cultures. RPMI media is usually supplemented with fetal bovine serum (FBS) at levels of 1 to 10% to supplement growth factors necessary to sustain specific cell line processes. However, FBS supplemented media presents some issues when there is a desire to perform routine quantitative analysis of the media. The high serum protein contents in the mixed media may interfere with standard chromatography columns for amino acid analysis. Removal of the proteins with precipitation may be required to prepare the samples before analysis with traditional chromatographic platforms. This additional sample prep may affect amino acid recoveries resulting in biased results with conventional analytics approaches for media analysis.

THE EXPERIMENT

A commercially available RPMI media (without glutamine) and cell culture grade FBS (USA origin) were mixed at the listed levels below. All media samples were handled following the manufacturer's instructions. Final solutions were diluted 10x before analysis on the Rebel with no additional sample preparation. (Figure 1)

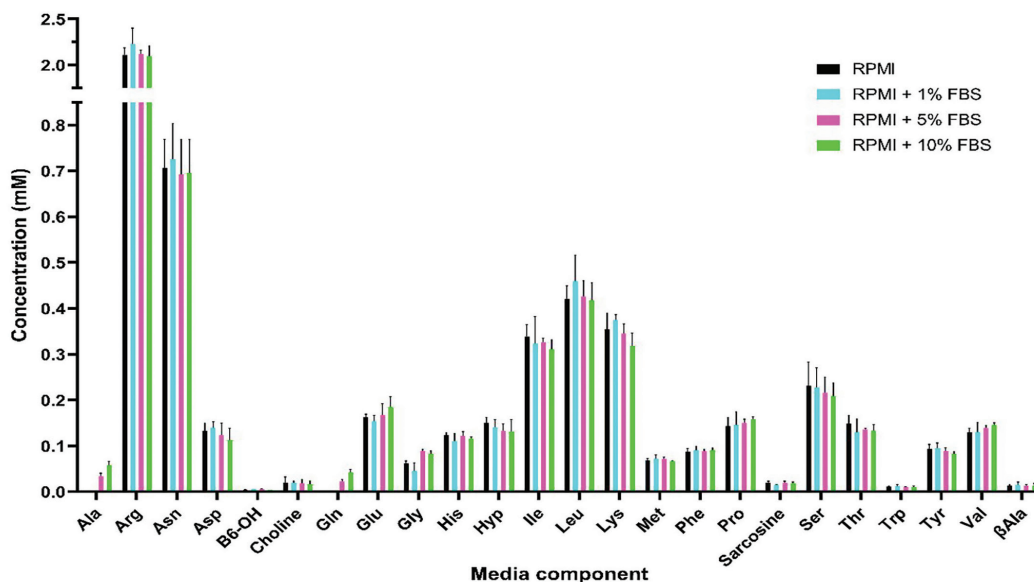


Figure 1: Media component concentrations from RPMI and RPMI with FBS supplementation. Error bars are from the standard deviation of n = 6 replicates.

DISCUSSION

The analysis was able to quantitate 24 different media components, including 19 amino acids, at a single dilution level. There were virtually no differences in the media components' concentrations between the base RPMI media and the RPMI samples with up to 10% FBS added. The exceptions to this observation were both Ala and Gln. Both Ala and Gln were not in the original formulation of RPMI. They were not detected in the base media and 1% FBS-supplemented media. However, in 5% and 10% FBS, both Ala and Gln were identified, which were consistent with the separate observation of detecting both these amino acids in pure serum tested at a 10x dilution (not shown). Corresponding to the formulation, Arg had the highest levels of all amino acids averaging 2.108 mM. In comparison, Trp had the lowest concentration with an average of 0.011 mM. With the Rebel, one can quickly and confidently screen serum-supplemented media formulations to ensure batch-to-batch media consistency before introducing the cell media to cultures.

