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LETTER TO THE EDITOR

Inability to Culture *Pneumocystis jirovecii*

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In their 2014 research article, Verena Schildgen et al. reported that *Pneumocystis jirovecii* can be successfully cultured and propagated from *P. jirovecii*-positive bronchoalveolar lavage (BAL) patient samples by using a permanent three-dimensional air-liquid interface culture system formed by CuFi-8 cells, a differentiated pseudostratified airway epithelial cell line (1). BAL samples from patients which were positive for *P. jirovecii* by quantitative PCR (Q-PCR) were inoculated into the air-liquid interface culture system formed by CuFi-8 cells. The cells and basal medium were harvested and tested for *P. jirovecii* using Q-PCR targeting the mitochondrial large-subunit (mtLSU) rRNA as well as the major surface glycoprotein (*msg*) genes after 5 days of culture. The results revealed an increase in mitochondrial genome equivalents from 10^2 to 10^7 and an increase in *msg* gene copy numbers of up to 10^3.

We were very interested in this paper and attempted to reproduce the results, given the previous inability to culture *Pneumocystis* and the potential benefit that this would have to our research agenda. We set up cultures using 10 BAL samples which were positive for *P. jirovecii* by Q-PCR using *msg* gene primers (2). We followed the protocol exactly as indicated in the paper, using CuFi-8 cells, which we obtained at an early passage number from the University of Iowa (the same source as reported in the paper) and used the same media as described in the paper. We were in communication with the senior author of the paper to be certain that we were utilizing the same methodologies. We used the *msg* Q-PCR (performed in our clinical microbiology laboratory; a cycle threshold [C_T] of ~20.6 represents 10,000 *msg* copies/reaction) to quantitate the organism load in cells growing in the air-liquid interface after 5 days of culture, as well as in the basal medium of the insert. In 8 out of 10 cultures with low starting organism burdens, as indicated by high C_T values, there was no detectable *Pneumocystis* DNA after 5 days of culture in either the cells or the basal medium. In the 2 BAL samples which had the highest organism loads based on C_T values by *msg* Q-PCR (20.96 and 21.23), organisms could be detected in the cells after 5 days of culture, but the C_T value (26.56 and 32.50, respectively) was higher (and the organism load was lower) than that of the original BAL fluid; the basal medium for both was negative. Thus, in none of our culture attempts was there evidence that *Pneumocystis* proliferated; we did see a rapid drop in *Pneumocystis* counts as measured by *msg* Q-PCR in all the samples.

We are disappointed that we are unable to replicate the results, as the ability to culture *Pneumocystis* would be of major benefit to our work. At this point, we are not aware of other groups that have attempted to replicate the results. We are writing this letter to communicate our findings to the larger *Pneumocystis* research community, and we would be interested in hearing about the experience of other groups with this methodology.

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