Testing *Corynebacterium bovis* growth under tissue culture conditions

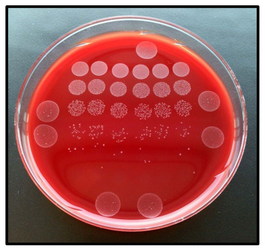
Alyssa Kleymann[1], Nick Zawadzki[2], Michael Fink[1,3] Lauren Habenicht[1,3] Derek Fong[1,3] Jori Leszcynski[1,3] Steven Anderson[3], Michael Schurr[2], Chris Manuel[1,3]

Anschutz

1. Office of Laboratory Animal Resources, University of Colorado Denver | Anschutz Medical Campus, Aurora, Colorado 2. Department of Immunology and Microbiology, and 3. Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO

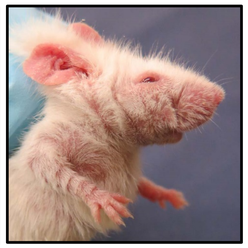
Corynebacterium bovis

- *Corynebacterium bovis* is an opportunistic bacterial pathogen that infects the skin of immunodeficient mice
- Immunocompetent mice are rarely impacted by *C. bovis*
- *C. bovis* has a negative impact of cancer mouse models
- 55% (38/69) of NCI's Cancer Centers have infected mice
- 57% (28/50) of the top 50 NIH Funded academic institutions have infected mice
- *C. bovis* is spread by equipment, supplies, and even frozen tumor tissue
- C. bovis is shed from infected mice which contaminate the vivarium environment
- *C. bovis* can survive on surfaces for >3.5 months
- Infected mice may appear either normal, scaly, or very scruffy and sick



***C. bovis* colonies on a 5% sheep-blood agar plate after 72 h**

Athymic nude mice naturally infected with *C. bovis* at CU Anschutz campus.

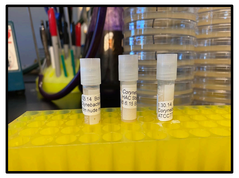


NSG mouse experimentally infected with *C. bovis* after 8 wk

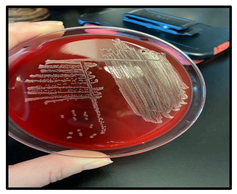
Hypothesis

- *C. bovis* will not grow in tissue culture media, or under tissue culture conditions.
  - This knowledge will diminish the risk of *C. bovis* infection transmission for tumor cell lines cultured in vitro.

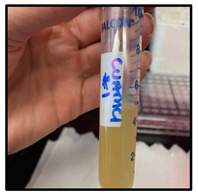
Materials and Methods



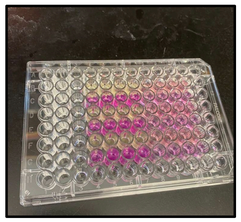
Isolates: CUAMC1, HAC, ATCC-7715



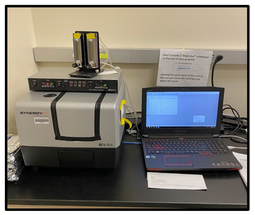
Bacterial Plate w/ Streaked Bacteria



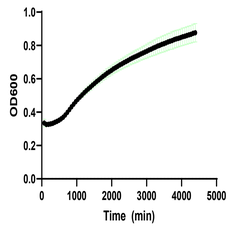
Overnight Culture in HIBTW



Microplate w/ cells + media



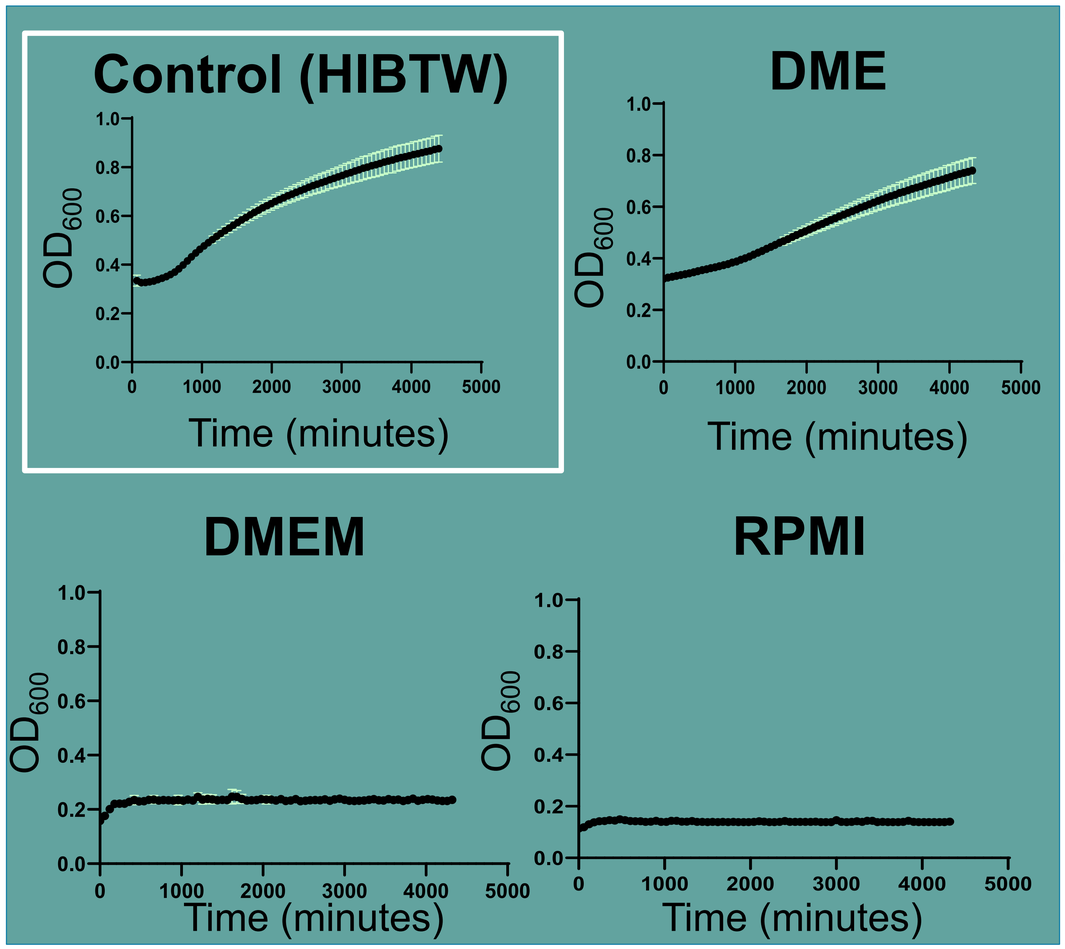
Biotek H1 Synergy Microplate Reader



Growth Curves

- Three *C. bovis* isolates, CUAMC1, HAC, and ATCC-7715 were grown under ideal liquid culture conditions in heart infused broth with 5% Tween 80 (**HIBTW**) at 32°C and with rotary shaking at 250 rpm for 24 h.
- To determine if *C. bovis* can grow under tissue culture conditions, 3 of the most common basal media used to grow human cancer cell lines were used including
  - DME/F12 +10% fetal bovine serum (**DME**)
  - DMEM/high glucose +10% FBS (**DMEM**)
  - RPMI 1640 +10% FBS (**RPMI**)
- One million CFU of each *C. bovis* isolate was cultured in each media using HIBTW as a positive control.
- Growth curves were generated using a Biotek Synergy automated incubator set to 37°C, 5% CO_2, without shaking and OD_{600} absorbance was recorded for each condition every hour for 72 h. These parameters reflect the most common conditions for tissue culture.

Growth Curve Results



Results and Discussion

- Under tissue culture conditions, *C. bovis* successfully grew in HIBTW (control).
- Unexpectedly, under the same conditions, all 3 isolates also grew in DME but failed to grow in DMEM and RPMI.
- Our data shows that *C. bovis* growth under tissue culture conditions is possible.
- These results highlight the importance of pathogen surveillance for tumor cell lines propagated in vitro and demonstrate the need for further investigation into *C. bovis* growth requirements.

Acknowledgements

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