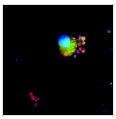

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Fluorescent in vitro model to assess adhesion and invasion of Bd in PAK

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1 Works for me dx.doi.org/10.17504/protocols.io.8ihhub6

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ABSTRACT

The largest current disease-induced loss of vertebrate biodiversity is due to chytridiomycosis and despite the increasing understanding of the pathogenesis, knowledge unravelling the early host-pathogen interactions remains limited. *Batrachochytrium dendrobatidis* (*Bd*)zoospores attach to and invade the amphibian epidermis, with subsequent invasive growth in the host skin. Availability of an *in vitro* assay would facilitate in depth study of this interaction while reducing the number of experimental animals needed. We describe a fluorescent cell-based *in vitro* infection model that reproduces host-*Bd* interactions. Using primary keratinocytes from *Litoria caerulea* and the epithelial cell line A6 from *Xenopus laevis*, we reproduced different stages of host cell infection and intracellular growth of *Bd*, resulting in host cell death, a key event in chytridiomycosis. The presented *in vitro* models may facilitate future mechanistic studies of host susceptibility and pathogen virulence.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Verbrugghe E, Rooij PV, Favoreel H, Martel A, Pasmans F (2019) *In vitro* modeling of *Batrachochytrium dendrobatidis* infection of the amphibian skin. PLoS ONE 14(11): e0225224. doi: 10.1371/journal.pone.0225224

MATERIALS

NAME	CATALOG #	VENDOR
Distilled Water		
HBSS with calcium and magnesium		
HBSS without calcium and magnesium		
Fetal bovine serum		
Paraformaldehyde	P6148	Sigma Aldrich
Leibovitz's L-15 Medium	11415049	Thermo Fisher
CellTracker™ Red CMTPX Dye	C34552	Thermo Fisher
CellTracker™ Green CMFDA Dye	C7025	Thermo Fisher
Hoechst 33342, Trihydrochloride, Trihydrate, 100 mg	H1399	Thermo Fisher
ProLong™ Glass Antifade Mountant	P36980	Thermo Fisher
Rat Tail Collagen Coating Solution	122-20	Sigma Aldrich

MATERIALS TEXT

24-well tissue culture plates
glass coverslips

1 **Prepare Cell Medium A:**
L15 medium: 70%
Distilled water: 20%
Fetal bovine serum 10%

2 **Prepare Cell Medium B:**
L15 medium: 40%
Distilled water: 55%
Fetal bovine serum: 5%

3 **Coat coverslips with Rat tail collagen:**
Add glass coverslips in a 24-well tissue culture plate. Coat the glass coverslips at 37°C for 2 hours. Therefore, carefully aspirate the Collagen Coating Solution and add 200 µl per well (so per coverslip). After 2 hours, rinse the coated surface twice with HBSS-. Coated tissue culture ware may be used immediately or air-dried and stored at 4 ^{o}C for up to one week.

4 **Staining of Primary Keratinocytes (PAK): (Work in the dark)**
- Add 1 ml of 3 µM Celltracker green CMFDA in cell medium A to the PAK
- Resuspend carefully
- Incubate for 30 min at 20°C 5% CO2
- Centrifuge for 5 min at 1500 rpm and 20°C
- Resuspend the pellet in 70% HBSS- and in meantime count the cells
- Centrifuge for 5 min at 1500 rpm
- Resuspend the cells in cell medium A to reach a concentration of 10e5 cells per mL
- Seed the cells at a concentration of 10e5 cells per well (so add 1 mL) which contains a collagen-coated glass coverslip
- Let the PAK attach for 1 hour at 20°C and 5% CO2
- Afther 1 hour, wash the cells with 70% HBSS+
- The cells are now ready to be exposed to *Bd* spores

IMORTANT NOTE: Include a control well that can be used to check the cells throughout the entire protocol via light microscopy.

5 ⚠

Staining of *Bd* zoospores: (Work in the dark)
- Isolate *Bd* zoospores and spin them down for 5 min at 3000 rpm (20°C)
- Remove the supernatant and resuspend them in 3 µM celltracker red CMPTX solution for 45 min at 20°C. Important here is that the 3 µM celltracker solution should be diluted in CELL MEDIUM B in order to ensure the motility of the zoospores.
- After 45 min, spin the spores down for 3 min at 3000 rpm
- Resuspend them in cell medium B and count the spores
- Dilute the spores to a concentration of 10e6 spores/ml in cell medium B

IMPORTANT NOTE: *Bd* zoospores lose their motility when exposed to cell medium A.
IMORTANT NOTE: Include a control well that can be used to check the motility of the spores throughout the entire protocol via light microscopy.

6 ***Bd* infection of PAK cells: (Work in the dark)**
- Add 1 ml of the spore suspension (= 10e6 spores) to the wells containing celltracker-labelled PAK cells (on a coverslip). As such the spores are seeded at a MOI of 10:1.
- Incubate for 2 hours at 20°C 5%CO2.
- After 2 hours gently wash the infected cells three times with 70% HBSS+ to remove non-adherent spores
- Replace cell medium B with cell medium A for another 2 hours to assess ADHESION or 22 hours to assess invasion

IMPORTANT: Use the control well to check the adhesion/invasion of the spores to/in the cells via light microscopy.

7 **Visualisation of early *Bd*-PAK interactions: (Work in the dark)**

ADHESION: 4 hours post infection (2 hours with cell medium B and 2 hours with cell medium A)
INVASION: 24 hours post infection (2 hours with cell medium B and 22 hours with cell medium A)

- Wash the infected cells 3 times with 70% HBSS+
- Fix the infected cells with 0.5 mL of 3% paraformaldehyde for 10 min
- Stain the nulear content with Hoechst
- Wash 2 times with 70% HBSS+
- Mount the coverslips using ProLong Gold antifade mountant
- Use fluorescence microscopy and confocal laser scanning microscopy to analyse *Bd*-PAK interactions