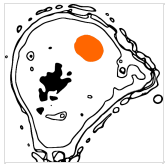

Version 1

Dec 15, 2020

Affinity purification of ookinetes in Petri dishes V.1

In 1 collection

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

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ABSTRACT

The ookinetes, as they come from the ookinete culture, are suspended in a solution that also contains blood cells and other parasite stages. Often, it is necessary to purify the ookinetes from these other cell types. This is achieved exploiting biological, chemical, and physical properties of the ookinetes that differ from the other cell types present in the ookinete culture.

This protocol exploits the adhesive and invading characteristics of ookinetes by allowing them to attach to a surface coated with Extracellular Matrix gel (ECM gel). The rest of the cells present in the ookinete culture do not have adhesion properties and can be simply washed away. ECM gel is composed of approximately 1800 proteins, the most abundant are type IV collagen, laminin, entactin, and other proteoglycans. The *Plasmodium* ookinete has several surface proteins that bind to the ECM gel components, such as the P25, and P21/28 protein families that bind to laminin and collagen, and the CTRP, SOAP, and WARP surface or secreted proteins that bind to laminin, and other glycosaminoglycans.

The purification in Petri dishes allows the obtention of a higher amount of ookinetes with the expense of being less pure than the purifications on coverslips. The ookinetes can be re-suspended by the use of dispase II, a protease that cleaves type IV collagen and repurposed for experiments that require them in suspension or to perform organelle and macromolecule extracts.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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COLLECTIONS

Culture and purification of Plasmodium berghei ookinetes

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Culture and purification of Plasmodium berghei ookinetes

DISCLAIMER:

This work involves working with mouse blood.

ABSTRACT

The ookinetes, as they come from the ookinete culture, are suspended in a solution that also contains blood cells and other parasite stages. Often, it is necessary to purify the ookinetes from these other cell types. This is achieved exploiting biological, chemical, and physical properties of the ookinetes that differ from the other cell types present in the ookinete culture.
This protocol exploits the adhesive and invading characteristics of ookinetes by allowing them to attach to a surface coated with Extracellular Matrix gel (ECM gel). The rest of the cells present in the ookinete culture do not have adhesion properties and can be simply washed away. ECM gel is composed of approximately 1800 proteins, the most abundant are type IV collagen, laminin, entactin, and other proteoglycans. The *Plasmodium* ookinete has several surface proteins that bind to the ECM gel components, such as the P25, and P21/28 protein families that bind to laminin and collagen, and the CTRP, SOAP, and WARP surface or secreted proteins that bind to laminin, and other glycosaminoglycans.

The purification in Petri dishes allows the obtention of a higher amount of ookinetes with the expense of being less pure than the purifications on coverslips. The ookinetes can be re-suspended by the use of dispase II, a protease that cleaves type IV collagen and repurposed for experiments that require them in suspension or to perform organelle and macromolecule extracts.

BEFORE STARTING

Equipment and materials:
1.- Petri dishes 60 mm in diameter.
2.- 10-100 and 200-1000 µl pipettes and tips.
3.- Incubator at 20°C and at 37°C.
6.- Refrigerated centrifuge at 4°C.
5.- 15 ml conical-bottom centrifuge tubes.

Reagents:
1.- Extracellular Matrix gel (ECM gel or Matrigel).
2.- ECM buffer: 30 mM NaCl, 4% sucrose, and 40 mM Tris-base at pH 8 (Mannuzza, et al., 2004).
3.- Phosphate buffered saline (PBS).
4.- Dispase II solution at 2.4 U/ml.
5.- EDTA solution at 0.5 M.
4.- Ookinete culture medium: RPMI 1640 medium at pH 8.3 supplemented with 23.81 mM sodium bicarbonate,

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0.37 mM hypoxanthine, 25 mM HEPES, 5000 U/ml penicillin, 5 mg/ml streptomycin, 10 mg/ml neomycin, and 20% heat-inactivated fetal bovine serum (Rodríguez, et al., 2002).
5.- Oocyst culture medium (optional): Schneider's *Drosophila* medium at pH 6.8 supplemented with 15.87 mM sodium bicarbonate, 20 mM HEPES, 3.68 mM hypoxanthine, 44 µM *para*-aminobenzoic acid, 0.2% lipid/cholesterol solution (Gibco), 100 u/ml penicillin, 100 µg/ml streptomycin, 200 µg/ml gentamycin, and 15% heat-inactivated foetal bovine serum (Al-Olayan, et. al., 2002).

Frank J. Mannuzza, Paula Flaherty, Stephen R. Ilsley, Martin L. Kramer (2004). Coated membrane for assessing the invasive capacity of a cell. US Patent.
https://patents.google.com/patent/US6740501

Rodríguez MC, Margos G, Compton H, Ku M, Lanz H, Rodríguez MH, Sinden RE (2002). Plasmodium berghei: routine production of pure gametocytes, extracellular gametes, zygotes, and ookinetes. Experimental parasitology.

Al-Olayan EM, Beetsma AL, Butcher GA, Sinden RE, Hurd H (2002). Complete development of mosquito phases of the malaria parasite in vitro. Science (New York, N.Y.).

Ookinete purification

1 Thaw the ECM gel overnight at **4 °C** in ice

Keep the ECM gel in ice at all times. ECM gel starts to form a gel at 20°C

2 Dilute the ECM gel 1:2 or 1:3 with cold ECM buffer, mix well and coat the Petri dishes with **0.5 mL** .
Allow gel formation at **37 °C** for **00:30:00** .

Up to 1:9 ECM gel dilutions can be made with the expense of reducing the ookinete yield.
Optionally, the coated Petri dishes can be left at 37°C until the ECM gel is dry, after which the coverslips can be stored at 4°C. Before use re-hydrate the ECM gel by adding ookinete medium for 30 min at 37°C.

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3 Add between **2.5 mL** to **6 mL** of ookinete culture to the Petri dishes and incubate for **04:00:00** at **20 °C** .

During incubation, the dishes can be gently shaken to increase the likelihood of contact between the ookinetes and the substrate.

4 15 min before the incubation ends, tilt the Petri dishes at a 10-15° angle. This will cause the sedimented cells to slide to one side of the container leaving most of the dishes already free of contaminant cells.

5 Remove the contaminant cells from one side of the dish and add **2 mL** of PBS from the other side of the dish to create a one-way flow of liquid.
Repeat the washes 6 or 8 times.
Add ookinete or oocyst medium for further cultivation.

Ookinete resuspension

6 Alternatively, the ookinetes can be brought back to suspension by digesting the ECM gel with dispase II.
After purification, instead of adding culture medium, add **2 mL** of dispase II and incubate for **00:30:00** at **20 °C** .

7 Add EDTA at a final concentration of **2 Milimolar (mM)** and mix well.

8 Tilt the Petri dish and transfer the ookinetes into a 15 ml conical-bottom centrifuge tube in ice.

9 Add **2 mL** of ice-cold PBS and pipette a couple of times to detach the remaining ookinetes.
Repeat this process 4 times.
Collect the ookinetes and the washings in the same tube.

10 **720 rcf, 4°C, 00:05:00** , remove the supernatant and repeat.

11 Re-suspend the pellet in **1 mL** ookinete or oocyst medium and count the number of ookinetes in a Neubauer chamber.

Mosquito infections

12

This method can be used to infect mosquitoes with purified ookinetes. However, the number of ookinetes needs to

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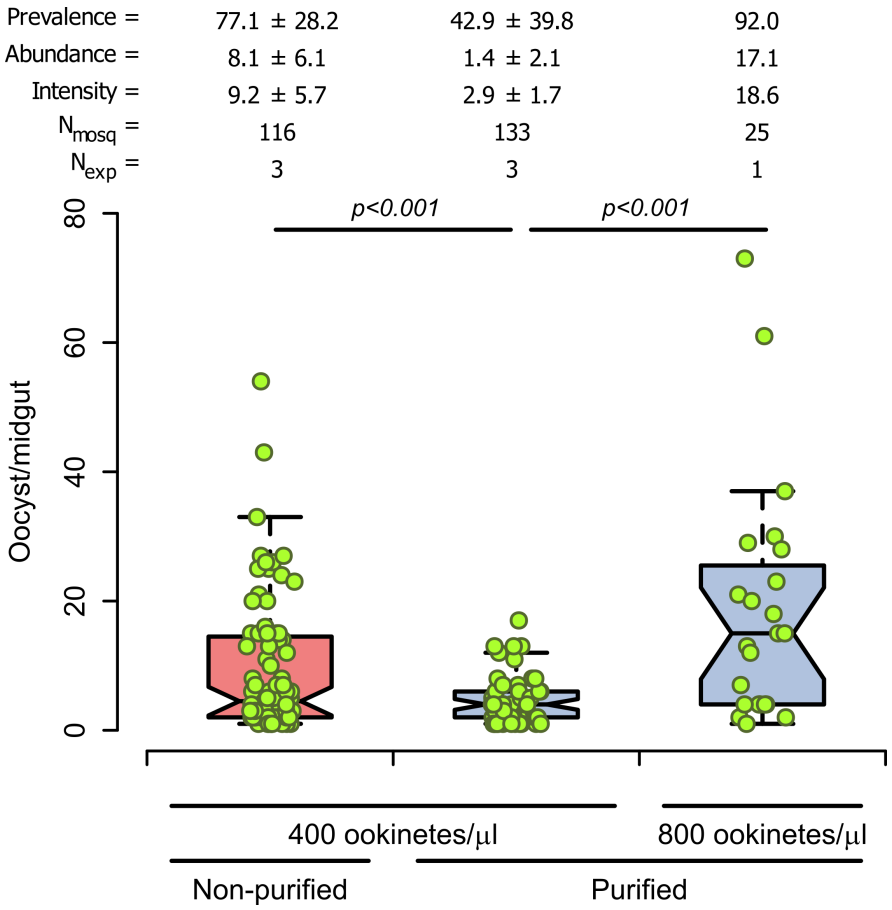
be increased in order to obtain equivalent infection rates compared to mosquito infections with non-purified ookinete cultures.



Infection parameters and abundance distribution of *Anopheles albimanus* mosquitoes fed with purified and non-purified GFP-expressing ookinetes. The ookinete cultures were split in half to form the purified and non-purified groups. The purified group was obtained following this protocol and resuspended with dispase II. The groups were brought to the same density of ookinetes and a third group was included where the number of ookinetes was doubled. The hematocrit was adjusted to 5% with the blood of an uninfected mouse to make the feeding treatments equivalent. Mosquitoes were fed with glass feeders for 30 min and then placed at 20°C with an *ad libitum* supply of 8% sucrose and 0.05% PABA. The mosquitoes were dissected at 72 hours post-feeding to determine the presence of oocysts in the midgut through epifluorescence microscopy. Dots represent a single mosquito, the box is the interquartile region, the middle line denotes the median, the notches are the 95% confidence interval of the median, and the whiskers illustrate the range of the data excluding the extreme points. *P* values obtained by performing a Kruskal-Wallis rank sum test followed by Pairwise Wilcoxon rank-sum test.

This result represents an improvement of what was observed by Munderloh U.G., et al., (1987) with ookinetes purified by Percoll gradients. While Munderloh U.G., et al., (1987) estimated that 110 ookinetes per µl were needed to obtain each oocyst in feedings with enriched ookinete cultures, and 510 ookinetes per µl to obtain each oocyst in feedings with Percoll-purified ookinete cultures, representing a 5-fold reduction in infectivity, we obtained an estimate of 43 ookinetes per µl per oocyst formed in feedings with non-purified ookinete cultures, and 138 ookinetes per µl per oocyst formed in feedings with purified ookinete cultures, representing a 3-fold reduction in infectivity. Likewise, the infection prevalence obtained here with purified ookinetes was, on average, 10% higher in comparison to the cited study, while the infection prevalence obtained with non-purified ookinetes was approximately the same.
These results are also comparable to those obtained by Sinden R.E., et al., (2007) with non-purified ookinetes.

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Sinden RE, Dawes EJ, Alavi Y, Waldock J, Finney O, Mendoza J, Butcher GA, Andrews L, Hill AV, Gilbert SC, Basáñez MG (2007). Progression of Plasmodium berghei through Anopheles stephensi is density-dependent. PLoS pathogens.
https://doi.org/10.1371/journal.ppat.0030195

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