BACTERIAL CROWDING – UPO Sevilla

QUALITATIVE | **CAPILLARY ASSAY USING MICROSCOPE**

1. ***E. Coli*** **inocula in triptone broth in low shaking (between 100-200 rpm/min)**

   Inocula must be incubated at low temperature and low shaking in order to achieve a peak development of flagella. High shaking might provoke the loss of flagella; the production of flagella wouldn't be possible in rich environments since bacteria wouldn't need them.

2. **Taking a sample from one inoculum and looking at the motility under the microscope.**

   It is crucial to observe that flagella have been properly developed in the bacteria; in the same way it is also important to see that those bacteria are mobiles. If the final result is a low motility, the assay should be restarted.

3. **Diluting the *E. Coli* culture 100 times.**

   We dilute the triptone broth in E. Coli culture a hundred of times. Incubate again at 30ºC in low shaking till exponential medium phase. The final volume would be 20 ml so we will have to inoculate 200 μl.

4. **Check motility under the microscope. Again, we check the motility to observe that it still exits.**

5. **Wash twice the culture centrifugating and resuspending in chemotaxis buffer.**

   It's essential to be really careful when resuspending pellets, it is needed to hit softly in the base of the tube. The centrifuges must be done in a low speed to prevent the loss of flagella (10 min in 8000 G)

6. **Measure optical density and adjust the volume in order to achieve around 10^7 cfu/ml (optical density ~0,01).**

1. **Set up the assay**

   Two capillaries are put over a microscope slide which will hold up a cover slip. Then we insert the bacterial dilution between the slide and the cover slip. Two new capillaries are inserted between the slide and the cover slip, inside of the bacterial dilution. One of those capillaries would contain a chemoattractant while the other one would be the control. Under a microscope we could see the difference between both capillaries and we would definitely be able to observe if there is chemotaxis toward this chemoattractant. The capillary located at the end is heated sealed meanwhile the chemo attractant is sucked.

   It's possible to look at the microscope slide under the microscope in different times and observe the diverse quantity of bacteria in the chemotactic capillary and in the control capillary.

8. Dilution and spread (optional)

After the incubation, the quantity of bacteria in those capillaries might be quantified when spread in plates. For that it is necessary to empty previously the capillary by means of centrifugation: The capillary content is obtained in a 1.5mL tube by centrifugation: Wash the outside of capillaries using distilled water and then break the seal and empty capillaries into 1.5mL tubes that contain 200 µl of chemotaxis buffer; after that , centrifuge it.

IMPORTANT: To work with *Pseudomonas* it is necessary to set up inocula in minimal medium instead of triptone broth. Plus, the culture must rest till it achieves the exponential late phase, since it is in here when flagella are developed. At the end of this assay the final incubation must be done at 30ºC.

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QUALITATIVE

AGAR SOFT PLATES

1. Prepare the soft agar follow this way:

a) Mix and autoclave (for 1 liter; makes ~ 40 plates):

Difco Bacto-Agar..... ~2-3 gm

NaCl ..... 5 gm

H_2O ..... 1000 ml

b) After autoclaving, cool to 60°C and add required amino acids, succinate, antibiotics (use at one-half the concentration used in solid plates and liquid media).

3. Mix vigorously and pour 20-25 ml/plate (10 cm diameter plates); allow plates to harden for at least 4 hours at room temperature before use.

4. Using minimal swarm plates, to insert a colony into the agar and then incubate it at 30ºC.

BACTERIAL CROWDING – UPO Sevilla

QUANTITATIVE

Capillary Assay in tip chambers

1. ***E. Coli*** **inocula in triptone broth in low shaking (between 100-200 rpm/min)**

   Inocula must be incubated at low temperature and low shaking in order to achieve a peak development of flagella. High shaking might provoke the loss of flagella; the production of flagella wouldn't be possible in rich environments since bacteria wouldn't need them.

2. **Taking a sample from one inoculum and looking at the motility under the microscope.**

   It is crucial to observe that flagella have been properly developed in the bacteria; in the same way it is also important to see that those bacteria are mobiles. If the final result is a low motility, the assay should be restarted.

3. **Diluting the *E. Coli* culture 100 times.**

   We dilute the triptone broth in E. Coli culture a hundred of times. Incubate again at 30ºC in low shaking till exponential medium phase. The final volume would be 20 ml so we will have to inoculate 200 μl.

4. **Check motility under the microscope. Again, we check the motility to observe that it still exits.**

5. **Wash twice the culture centrifugating and resuspending in chemotaxis buffer.**

   It's essential to be really careful when resuspending pellets, it is needed to hit softly in the base of the tube. The centrifuges must be done in a low speed to prevent the loss of flagella (10 min in 8000 G)

6. **Measure optical density and adjust the volume in order to achieve around 10^7 cfu/ml (optical density ~0,01).**

7. **Setting up the assay.**

   We spread 22mL of the bacteria dilution in each well in a tip empty sterilized chamber. Aside, we take a sample of inocula and dilute it 10^5 times and spread it in order to quantify the number of bacteria. Once the chemotactic chamber is ready, we place some syringe's needles in each cubicle filled with either the chemoattractant or the repellent we want to study, plus the appropriate control at 30ºC. Leaving the chemotactic chamber as horizontal and immobile as possible.

8. **Dilution and spread in LB plates using the content of capillaries**

   Dilute the content of capillaries (we use 2 10^2 and 2 10^4 dilution factor). Spread in LB plates and incubate it at 37ºC overnight. Also, you could quantify bacteria which are inside of capillaries by fluorescent microscope supposing those

bacteria have any fluorophore.

Important. On the one hand, for this assay, we have been using two different kinds of capillaries, both syringe's needles and micropipettes tips. On the other hand, we have also used two diverse types of chamber, principally and more often tip chamber, but occasionally needle's cups or heated sealed 10μl tips.
To work with *Pseudomonas* it is necessary to set up inocula in minimal medium instead of triptone broth. Plus, the culture must rest till it achieves the exponential late phase, since it is in here when flagella are developed. At the end of this assay the final incubation must be done at 30ºC.

BACTERIAL CROWDING – UPO Sevilla

QUANTITATIVE | **CAPILLARY ASSAY USING 96-WELL PVC MICROPLATES**

1. ***E. Coli*** **inocula in triptone broth in low shaking (between 100-200 rpm/min)**

Inocula must be incubated at low temperature and low shaking in order to achieve a peak development of flagella. High shaking might provoke the loss of flagella; the production of flagella wouldn't be possible in rich environments since bacteria wouldn't need them.

2. **Taking a sample from one inoculum and looking at the motility under the microscope.**

It is crucial to observe that flagella have been properly developed in the bacteria; in the same way it is also important to see that those bacteria are mobiles. If the final result is a low motility, the assay should be restarted.

3. **Diluting the** ***E. Coli*** **culture 100 times.**

We dilute the triptone broth in E. Coli culture a hundred of times. Incubate again at 30ºC in low shaking till exponential medium phase. The final volume would be 20 ml so we will have to inoculate 200 µl.

4. **Check motility under the microscope. Again, we check the motility to observe that it still exits.**

5. **Wash twice the culture centrifugating and resuspending in chemotaxis buffer.**

It's essential to be really careful when resuspending pellets, it is needed to hit softly in the base of the tube. The centrifuges must be done in a low speed to prevent the loss of flagella (10 min in 8000 G)

6. **Measure optical density and adjust the volume in order to achieve around 10^7 cfu/ml (optical density ~0,01).**

7. **Assay set-up**

1 µl capillary pipettes would be used as a capillary. One of its ends will be heat sealed. At the same time, the unsealed end will suck the chemo attractant; Whereas the control capillary won't suck the chemo attractant but chemotaxtis buffer.

As for the chemotactic chamber, 96-well PVC microplates will be used. Each well will be filled with some bacterial dilution of 200µL. In order to maintain capillaries still agarose gel 2% will be used; that is to say, this gel will be put above

the 96-well PVC micro plate and will be pierced by the capillaries.

8. Incubation at 30ºC for 1h.

9. Dilution and spread in LB plates.

Once the capillaries have been taken out from the gel, the dilution and spread in LB plates is started. The capillary content is obtained in a 1.5mL tube by centrifugation: Wash the outside of capillaries using distilled water and then break the seal and empty capillaries into 1.5mL tubes that contain 200 µl of chemotaxis buffer; after that , centrifuge it. LB plates are incubated overnight.

IMPORTANT: To work with *Pseudomonas* it is necessary to set up inocula in minimal medium instead of triptone broth. Plus, the culture must rest till it achieves the exponential late phase, since it is in here when flagella are developed. At the end of this assay the final incubation must be done at 30ºC.

BACTERIAL CROWDING – UPO Sevilla

QUANTITATIVE | **CAPILLARY ASSAY USING FLOW-CHAMBERS**

1. ***E. Coli*** **inocula in triptone broth in low shaking (between 100-200 rpm/min)**

   Inocula must be incubated at low temperature and low shaking in order to achieve a peak development of flagella. High shaking might provoke the loss of flagella; the production of flagella wouldn't be possible in rich environments since bacteria wouldn't need them.

2. **Taking a sample from one inoculum and looking at the motility under the microscope.**

   It is crucial to observe that flagella have been properly developed in the bacteria; in the same way it is also important to see that those bacteria are mobiles. If the final result is a low motility, the assay should be restarted.

3. **Diluting the** ***E. Coli*** **culture 100 times.**

   We dilute the triptone broth in E. Coli culture a hundred of times. Incubate again at 30ºC in low shaking till exponential medium phase. The final volume would be 20 ml so we will have to inoculate 200 µl.

4. **Check motility under the microscope. Again, we check the motility to observe that it still exits.**

5. **Wash twice the culture centrifugating and resuspending in chemotaxis buffer.**

   It's essential to be really careful when resuspending pellets, it is needed to hit softly in the base of the tube. The centrifuges must be done in a low speed to prevent the loss of flagella (10 min in 8000 G)

6. **Measure optical density and adjust the volume in order to achieve around 10^7 cfu/ml (optical density ~0,01).**

7. **Assay set-up**

   - *Sterilize two flow chambers using UV.*
   - *Fill 7 capillaries with chemotaxis buffer and other 7 with the chemoattractant.*
   - *Fill the flow-chamber channel with 100µl of cell suspension. Put two capillaries in each channel, one with chemoattractant and the other with chemotaxis buffer.*
   - *Spread $2x10^3$, $2x10^4$ and $2x10^5$ dilutions over LB+Ap plates.*

- *Put 200µl of suspension into the Adler chamber. Place capillaries over it and look at under the microscope at t=0,15,20,45 y 60.*

8. Incubation at 30ºC for 1h.

9. Dilution and spread in LB plates.

Once the capillaries have been taken away from the gel, the dilution and spread in LB plates is started capillaries (we use 2 10^3, 2 10^4 and 2 10^5 dilution factor). The capillary content is obtained in a 1.5mL tube by centrifugation: The capillary content is obtained in a 1.5mL tube by centrifugation: Wash the outside of capillaries using distilled water and then break the seal and empty capillaries into 1.5mL tubes that contain 200 µl of chemotaxis buffer; after that , centrifuge it. LB plates are incubated overnight.

IMPORTANT: To work with *Pseudomonas* it is necessary to set up inocula in minimal medium instead of triptone broth. Plus, the culture must rest till it achieves the exponential late phase, since it is in here when flagella are developed. At the end of this assay the final incubation must be done at 30ºC.