

Daily Culturing Routine and Culture Dilution

Reagents:

- Sterile Hood
- Complete Medium
- Parasite Culture
- Culture Gas: 1% oxygen, 3% carbon dioxide, 96% nitrogen.

Protocol:

- 1) Remove the culture flask carefully from the incubator without disturbing the sediment cells and move it to the sterile hood.
- 2) Remove the old medium by aspiration taking care not to remove the cells.
- 3) Smear the stock (10mL) or large (25mL) culture by taking a drop of cells using a sterile pipette. Calculate the culture parasitemia by Giemsa staining and microscopic inspection (See section Giemsa Staining of Cultures).
- 4) Add 37°C complete medium to the stock culture (10mL) or large culture (25mL).
- 5) Gas each culture ~30" using culture gas mixture. Also gas the complete medium to maintain proper pH. Visually check the complete medium and cultures daily for traces of bacteria/fungal growth.
- 6) Return the culture to incubator unless dilution is required (see next step). Make sure the culture cap is tightly closed.
- 7) Each stock and large flask should be diluted 1:10 when they reach $\geq 10\%$ parasitemia. It usually takes 3-4 days after dilution to 1% for the culture to reach ~10% parasitemia again. To dilute, resuspend the culture and transfer x mLs of it into a new flask where:

$$x = \text{volume of new culture} \cdot \text{final parasitemia required} / \text{initial parasitemia}$$

$$\text{Example 1: } x = 10 \cdot 1 / 10 = \underline{1\text{mL}}$$

$$\text{Example 2: } x = 25 \cdot 1 / 10 = \underline{2.5\text{mL}}$$

- 8) Add fresh blood to the new culture. Grow cultures @ ~5% haematocrit. Since the blood used is washed and then diluted to 50% using complete medium, to obtain a 5% haematocrit culture, add one tenth the volume of the new culture fresh washed blood.

$$\text{Example 1: For a 10mL stock culture} = \text{Add 0.5mL of washed blood}$$

Example 2: For a 25mL stock culture = Add 2.5mL of washed blood

- 9) Add 37°C complete medium to make of the remainder of the volume of the new culture.

Example 1: For a 1:10 dilution on a 10% parasitemia 10mL stock...

1mL stock culture
1mL washed blood
8mL warm complete medium
10mL total new culture

Example 2: For a 1:10 dilution of a 10% parasitemia 10mL stock to a new large 25mL culture...

2.5mL stock culture
2.5mL washed blood
20mL warm complete medium
25mL total new culture

- 10) Gas the new cultures for ~30" using culture gas and return to the incubator. Make sure the culture flask caps are tightly closed.