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 ≥ 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 = volume of new culture • final parasitemia required / initial parasitemia

   Example 1: **x** = 10 • 1 / 10 = <u>1mL</u>

   Example 2: **x** = 25 • 1 / 10 = <u>2.5mL</u>

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.

   Example 1: For a 10mL stock culture = 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
<u>8mL warm complete medium</u>
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
<u>20mL warm complete medium</u>
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.