**Culturing Crithidia fasciculata in brain heart infusion medium**

Brain Heart Infusion (BHI)

Sigma 53286-500G

37 g/L in dH2O

autoclave

Hemin from bovine >90%

Sigma H9039-1G

2.5 mg/ml in 50 mM NaOH

 400 ml dH2O

 1600 mg NaOH pellets

 1 g hemin

Filter sterilize or autoclave

Aliquot and store at -20 °C.

Complete media

4 ml 2.5 mg/ml hemin in 500 ml BHI (20 µg/ml hemin final)

Counting cells

Dilute cells 1:10 with 3% formalin and stain

Add stain last

 25 µl formalin

 25 µl cells

 200 µl stain

Stain

NaCl 4 g

EDTA 0.1 g

Genetian Violet 0.75 ml

dH2O to 500 ml

Load 10 µl on hemacytometer

Count cells in 5 squares of 25-square grid:

 **A** X X X **B**

 X X X X X

 X X **C** X X

 X X X X X

 **D** X X X **E**

Calculate cells/ml

Cells/ml = A + B + C + D + E **x** 25 **x** 104 **x** 10 (dilution factor)

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We typically maintain cultures between 1 x 105 cells/ml and 1 x 108 cells/ml. We split them 100-200X every other day and 200-500X for a weekend.