Verification of the efficiency of killing *Trypanosoma brucei* by autoclaving

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Aim: In many laboratories trypanosome cultures are routinely considered to have been inactivated, and all organisms killed, by either treatment with hypochlorite (branded as Chloros) or by heat treatment (autoclaving). The purpose of this report is to verify that autoclaving results in efficient killing.

Date of test: 12.07.04

Test carried out by: Dr Clare Allen.

Procedure: To confirm the killing of *Trypanosoma brucei* by autoclaving, 10ml of bloodstream form *T. brucei* cells (concentration = 2.4 x 10^6/ml) were autoclaved in a 50ml glass Duran bottle. The discard cycle was used to treat parasite contaminated waste: a 2h cycle which reached 126°C main sequence, 124°C load, for 20mins under 20 psi / 1.4 bar pressure.

Prior to autoclaving, 20μl of this culture was spotted onto a glass slide and the culture examined. A representative image of the healthy motile cells was obtained on a light microscope using a Nikon DXM 1200 digital camera (Figure 1A). Post autoclaving the culture was again examined by microscopy. The culture was now found to contain cell

debris and immotile, rounded-up cells. 20μl of this autoclaved culture was spotted onto a glass slide and an image of the autoclaved cells was taken as before (Figure 1B). Critically, no motility was observed within the culture, confirming efficient killing.

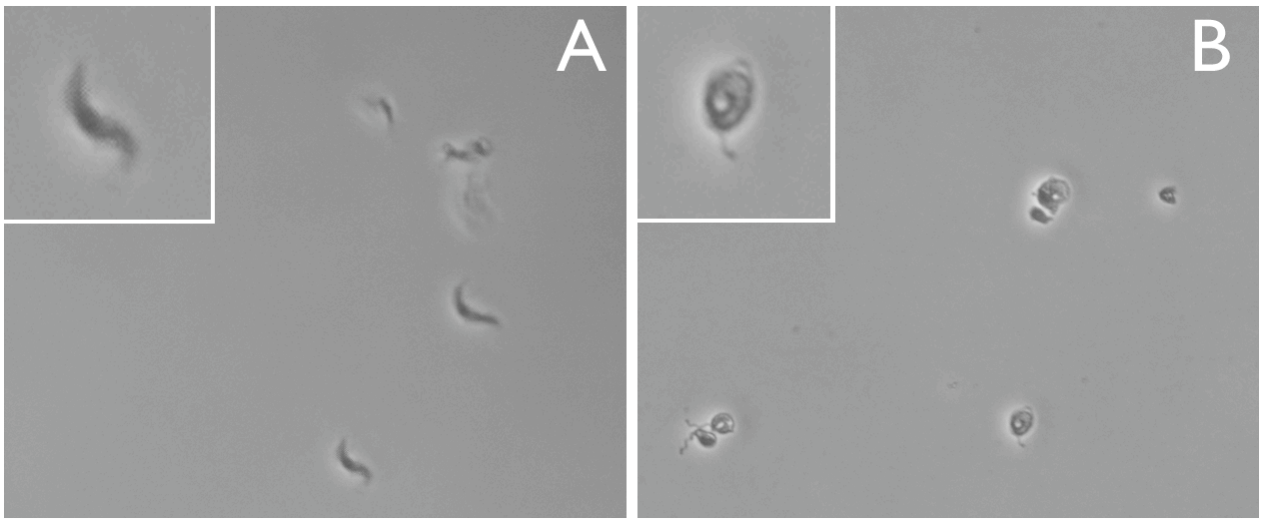

Figure 1: Phase contrast images of trypanosome cultures before and after autoclave treatment. Panel A: trypanosome culture prior to passage through the autoclave. Panel B: trypanosome culture post autoclaving. Insets in each panel are electronic enlargements of representative cells from each field.