

Verification of the efficiency of killing *Trypanosoma brucei* by TriGENE reagent

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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 Chlorox) or by heat treatment (autoclaving). The purpose of this report is to verify that TriGENE, an autoclave-compatible inactivation reagent efficiently kills trypanosomes at the doses indicated in many standard operating procedures.

Date of test: 08.09.05

Test carried out by: Ms Karen Wolfreys

Procedure: To confirm the killing of *Trypanosoma brucei* by TriGENE, 1ml aliquots of bloodstream form Lister 427 *T. brucei* cells (concentration = 1.0×10^6 /ml) were placed in 24 well culture dishes. TriGENE was added to the final concentrations in table 1 below. The wells were observed at time 0, 20

seconds, and 1 minute. They were then incubated at 37°C, 5% CO₂ and observed again at 10 minutes, 1 hour and after 4 hours.

Findings: *T. brucei* bloodstream form were killed at concentrations of 0.1% TriGENE and above within 20 seconds. Complete cell lysis was evident with concentrations of 1% and above at 20 seconds.

Conclusions: TriGENE disinfectant can be used at concentrations of 1% and above as an effective detergent to inactivate waste liquid and general laboratory ware for trypanosomes.

Table 1: Effect of TriGENE reagent on *T. brucei* cultures, ascertained for a range of times of exposure and final concentration of reagent. All observations were performed using log phase trypanosomes in complete HMI-9 medium. Viability is expressed as nn/mm, where nn is the percentage viable (motile) and mm is the percentage lysed (determined as clear loss of membrane integrity and cytosolic leakage).

Concentration (%)	Time (minutes)				
	0.35	1.0	10.0	60.0	240.0
Control (0)	100/0	100/0	100/0	100/0	100/0
0.001	100/0	100/0	100/0	100/0	0/0
0.01	100/0	100/0	100/0	100/0	0/20
0.1	0/24	0/50	0/75	0/95	0/95
0.25	0/50	0/75	0/100	0/100	0/100
0.5	0/100	0/100	0/100	0/100	0/100
1.0	0/100	0/100	0/100	0/100	0/100

Figure 1: Morphological effects of TriGENE on *Trypanosoma brucei* at various concentrations and times of exposure. Cultures were exposed to TriGENE as described in the text and images captures by phase contrast microscopy. Panel A: 0.001% TriGENE, 10 minutes exposure. Cells retain viability and are recognisable as trypanosomes. Panel B: 0.25% TriGENE, 10 minutes. Cells are denatured, clumped and inviable. Panel C: 10% TriGENE, 20 seconds. Panel D: 10% TriGENE, 10 minutes.

