Table 2

Marker of apoptosis	Example of assay used	Method of detection	Practical considerations	Relevance for malaria ecology
Fragmented DNA leading to the generation of fragments with 3'OH groups	*In situ* cell death detection kit, Flourescein (Roche)	DNA of fixed and permeabilized cells labelled by the addition of flourescein dUTP at strand breaks by terminal transferase. Flourescein then detected by fluorescent microscopy (figure 3)	– More laborious than using Acridine orange, CaspaTag or Anexin V detection – Gives clear unambiguous results. – Requires cells to be dead so cannot perform viability tests in conjunction. – Slides can be stored (at 5°C) for a few days for later analysis therefore large scale experiments possible	Good relevance as we would expect this process to be the same for mammalian and protozoan cells. However as DNA fragmentation is thought to be a late process in apoptosis may only see markers at a later time point than induction of apoptosis pathways.
Condensed chromatin	Acridine orange (Sigma)	Differentially stains SS and DS nucleic acids - enables the detection of condensed chromatin. Apoptotic cells should show an intense red staining in nucleus	– Quick and easy to use – False positives possible and Results not as clear as with TUNEL – Viability tests can be performed in conjunction – Large scale experiments possible	See above
Activation of caspase-like molecules	CaspaTag (Chemicon international, USA)	A fluorescent labelled general caspase inhibitor (FAM.VAD.fmk (green) and SR.DEVD.fmk (red)) binds to active caspase within the cell. Positive cells fluoresce under fluorescent microscope	– Quick and easy to use – Results not as clear as with TUNEL – Viability tests can be performed in conjunction – Large scale experiments possible	The role of caspase-like molecules controversial in protozoan parasites therefore it is not possible to be certain that apoptosis is being detected. However, if caspase molecules are a reliable marker they would be useful as an early marker of induction.
Translocation of phosphatidylserine to outer cell membrane	Annexin V- FITC apoptosis detection kit (Sigma, UK)	Positive display green annexin labelling on the cell surface, which can be detected by fluorescent microscopy	– Quick and easy to use – Results not as clear as with TUNEL – Viability tests can be performed in conjunction – Large scale experiments possible	May not be relevant for malaria cells for two reasons. 1. The cell membrane of protozoan parasites is very different to that of mammalian cells. 2. The ultimate reason for mammalian cells expressing phosphatidylserine on the outside of apoptotic bodies in order to be taken up by phagocytes, is not relevant for the mosquito midgut.
Morphological Markers e.g. membrane blebbing and formation of apoptotic bodies	Electron microscopy	Observation of cell morphology under electron microscope to detect membrane blebing or formation of apoptotic bodies	– Time consuming and expensive – Not a good basis for morphological changes seen in malaria apoptosis – Requires cells to be dead so cannot perform viability tests in conjunction. – Large scale experiments not possible but may be useful in conjunction with other assays	Malaria parasite cells differ in structural aspects from mammalian cells it is therefore not clear whether the structural changes observed in mammalian cells would be relevant for these parasites. The ultimate reasons for formation of apoptotic bodies to be taken up by macrophages also not relevant in the mosquito midgut.