Standard Operating Procedures.

Chemosensitivity testing *in vitro*.

Adherent cell lines: Cells which strongly adhere to tissue culture plastics are initially trypsinised from stock culture flasks and between 1 and 2 x 10^3 cells are placed into each well of a 96 well plate (U shaped wells) with each well containing a final volume of medium of 200 µl. Following an overnight incubation at 37°C in an atmosphere containing 5% CO_2 / 95 % air, all medium is removed and replaced with medium containing drug. Cells are exposed to a range of drug concentrations (8 wells per drug exposure). Each plate contains a blank (medium only / no cells) and a control (drug vehicle only). For **continuous** drug exposures, the plates are incubated for 5 days at 37 °C prior to assessing cell survival. For **timed** exposures, drug solutions are removed and the cells are washed twice with Hanks Balanced Salt Solution (HBSS, 200 µl per well per wash). Following washing, 200 µl of medium is added to each well and the cells incubated for 5 days at 37 °C.

Suspension cell cultures:

A) Timed exposures. Cells which do not attach to plastic culture plates are exposed to a range of drug solutions in universal tubes containing 5 ml of medium + drug. Following drug exposure, cells are centrifuged (1000 x g for 5 mins) and the pellet resuspended in HBSS. Following a further washing step, cells are resuspended in growth medium, counted using a haemocytometer and between 1 and 2 x 10^3 cells plated into each well of a 96 well plate as described above (ie 8 wells per drug exposure). Cells are then incubated for 5 days prior to chemosensitivity assessment.

B) Continuous exposure. Between 1 and 2 x 10^3 cells are plated into each well of a 96 well plate (180 µl cell suspension per well). Drug solutions at 10 times the desired final concentration are added to each well (20 µl drug per well, 8 wells per drug concentration), and the solutions mixed by gentle tapping of the plate. Plates are then incubated for 5 days.

Chemosensitivity testing using the MTT assay.

Following either a 5 day post drug exposure recovery period or a 5 day continuous exposure to drugs, 20 µl of MTT (5 mg ml^{-1}) is added to each well of the 96 well plate. Following a further 4 hour incubation at

37°C, medium is completely removed from each well (this applies only to adherent cell lines) and the formazan crystals dissolved in 150 µl DMSO per well. For suspension cultures, 200 µl of medium plus MTT is removed from each well (taking care not to disturb the formazan crystals) prior to the addition of 150 µl DMSO per well. Once the formazan has dissolved, the solution is mixed (using a spatula) and the absorbance of the resulting solution determined at 550 nm using a multi-well spectrophotometer. Cell survival is calculated from the mean absorbance of the treated plates (mean of 8 wells) divided by the mean absorbance of the control (mean of 8 wells) and the final result is expressed as percent cell survival taking the absorbance of the control cultures to be 100 % survival.