# PATHOLOGY CORE

## Making Cell Line Blocks

### Preparing a cell block from cell lines / cell suspensions

Cell lines or purified cell suspensions are a valuable tool to:

- provide known positive controls
- provide homogeneous tissue in unlimited amount, characterizable by molecular, cytogenetic and biochemical means
- provide models for ex-vivo cell cycle and activation-dependent effects

Cells grown in culture or obtained ex-vivo can be easily cytospun or smeared on a slide. However, conditions are quite different from a tissue block, fixed and embedded in paraffin.

To this end cell suspensions can be fixed, embedded in agar (an inert material), and processed as a piece of tissue

### Live cell preparation

- Prepare 1% Agarose, cell culture grade, in isoosmotic PBS. You need to boil to dissolve it in PBS. Check for loss of water as vapor during boiling and replace with distilled water
- Bring to approx 50 C°. The agar solidify below this point
- Prepare your cells as a cell suspension in minimal amount of medium, at room temperature
- To make a 0.5 ml agar block you may need between 10 to 20x10 6 cells. You can scale this down to as little as 0.5 x 10 6 in 50 µl but your cells will be very sparse on section
- Prepare in advance 1.5 ml Eppendorf tubes (or small PCR tubes for micro-preparations) to which you cut off and discarded the conical bottom (!). Cap the tube
- Mix evenly and thoroughly your cells with 0.5 ml of agar (or less) and very quickly transfer to the capped inverted tube. No bubbles! You may want also to cut the pipette tip, so you have a larger opening
- Place the still molten agar and tube on ice until is solid
- Then open carefully the cap, and gently extract the agar cylinder from its base (the cap). At this point you can cut the agar piece in two with a razor blade, freeze half and fix in formalin the other. Your cells are still alive and biochemically active at this point.

# PATHOLOGY CORE
## Making Cell Line Blocks

### Live cell preparation

- Proceed as above, but you fix the cells before. Then you can use Agarose in PBS, regardless of osmolarity
- Fixing the cells before embedding, prevents movement of labile antigens or loss of short-lived molecules

### Tricks

It is very convenient to have on the same block a positive and a negative control, but how to distinguish two semi-transparent block of agar and identical-looking cells? Put a tiny amount of India Ink (as your Surgical Pathologist) in the molten agar before embedding the cells. Must be not more than a faint grey hue, enough to distinguish the block with naked eye and at the microscope.