HTA Adequacy Study – cell counting methodology for SurePath LBC preparations

Please read this sheet before you start the cell counts. It is important to the study that all participants conduct the cell counts in the same way to allow a standardised approach and comparison of data.

The details of each count must be entered on the electronic Cell Counting Spreadsheet provided. The spreadsheet will ask for the FN value of your eyepieces (typically FN 22, 20 or 16); whether or not your microscope produces a 'true' or an inverted image; and the quadrant of the deposit you have selected to perform the counts (with slide label to the left). The ten counts are then recorded individually. Please follow these steps:

- Examine the slide naked eye. The deposit will often look completely even (Figure 1), but sometimes a paler arc may be noted at the edge (Figure 2). Very rarely part of the circumference of the deposit may look slightly darker. Choose a quadrant (12, 3, 6 or 9 o'clock) which is **neither hypo- nor hypercellular**. In Figure 2 the 3 o'clock position should therefore be avoided.

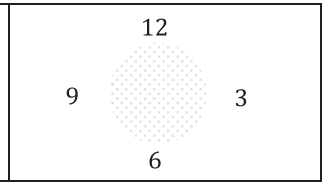
Figure 1	

Figure 2	

- The high power fields (x40 objective) used for counting cannot be preselected. Start at the edge of one quadrant of the deposit and work in towards the centre of the deposit. You may therefore be starting your count at the 12 o'clock, 3 o'clock, 6 o'c1ock or 9 o'clock positions and be counting in either a vertical or horizontal direction.

- Counts should be performed on 10 consecutive fields, working from the edge of the deposit towards the centre. Do not allow fields to overlap, but equally do not introduce gaps between fields. Do not pass over a field if it is either particularly hypo- or hypercellular. If a field is hypercellular, divide it into halves or quarters and try to remember as you go along what you have already counted.

- Only squamous cells with nuclei are counted but these can be of mature or parabasal / metaplastic type. Both single cells and cells in groups must be counted. Note that very pale nuclei if still visible are counted. Free nuclei are not counted. Anucleate squames / fragments of squamous cytoplasm are not counted. Syncytial aggregates of squamous cells as seen in cytolysis can be counted according to the number of nuclei they contain even if the cytoplasmic margins of individual cells are not identifiable.

- Cells at the edge of the field are counted if the entire circumference of the nucleus can be seen. If only part of the nucleus is visible do not count. Do not move the field to see cells at the edge.

- Counting must include cells on all planes of focus. When there are exceptionally thick groups of cells, which cannot be counted individually, an estimate of cellularity can be used. A full quadrant of a high power field contains approximately 1,000 small parabasals and approximately 750 mature squames. This figure can be scaled up or down to match the amount of the field covered e.g. a sheet of small para basal squamous cells covering half of the field would equate to approximately 2,000 cells.