Preparation of slides for the dilution studies

In this part of the study the impact of total cellularity and the relative proportion of abnormal cells on the screener's ability to correctly detect that abnormality is being examined. In the protocols we have committed to making a further 8 or 9 slides from selected positive cases and either diluting those samples (unmixed dilutions) or mixing them with varying amounts of a negative sample (mixed dilutions). The protocol reads as follows:

'A total of 180 SurePath™ and 180 Thin Prep® cases will be selected from material routinely accessioned at the Royal Liverpool University Hospital and the Manchester Cytology Centre, which display a range of histologically confirmed low and high grade cytological abnormalities. Following selection, the cases will be entered on the study database and anonymised to ensure compliance with the Human Tissue Act 2004. The cases will range from those containing plentiful hyperchromatic (darkly staining) dyskaryotic cells, to those which have scanty abnormal cells; are pale staining; show minimal nuclear changes or form microbiopsy fragments. The latter sub-types are known to cause diagnostic problems.

Eight preparations will be made from each sample. Serial dilutions with approximate cellularities of 5-10k, 10-15k, 15-20k, 20-25k, 25-35k, 35-45k, 45-55k and 55+k will be made from half of the cases. The range of dilutions is skewed towards preparations of lower cellularity as these are expected to have higher false negative rates. The remaining cases will be mixed with known negative cases in varying proportion to establish sets of slides containing <25, 25-49, 50-99, 100-149, 150-199, 200-399, 400-799, 800-1600 and 1600+abnormal cells. In total 3,000 new slides will be prepared. '

The methodology will have to be different for the two systems as the means by which the systems acquire cells is different. A pilot of 5 SurePath and 5 ThinPrep cases for the straight dilutional component of this part of the study should be performed and assessed fully before processing others.

The cellularity of the original slide can be determined by a cell count. Theoretically, at least for Surepath, we could tailor the dilutions to suit each sample. This would be unwieldy and could be very complicated for the technical staff. It is suggested therefore that a standard methodology is used for all cases accepting that there may be a greater spread of cellularities than is quoted in the original protocol. Assuming an average total cellularity of 60k (original slide) then the dilutions below would give approximate cellularities of 45k, 30k, 25k, 22.5k, 20k, 15k, 10k, 5k and 2.5k. The original slide together with the dilutions would provide a total of 10 slides for each case. Following the cell counts 8 slides, which best suit the required cellularities, will be selected for morphological assessment by the participating laboratories.

SurePath

- The residual samples are stored in tubes and have been 'topped up' with 2ml of collection fluid.

- The tube should be recentrifuged, drained and the cell deposit topped up with 1ml of collection fluid to produce a working solution. (The working solution will be diluted with collection fluid to produce specimens of varying cellularity.)
- The slides to be used in the PrepStain machine must be appropriately labelled with the case accession number followed by the dilution number to ensure traceability. (If the methodology is successful it may be easier in subsequent runs to complete the same dilution for a block of cases rather than having differing dilutions on a single run).
- The PrepStain machine will be used as normal but there will be no samples in the sample test tube rack; rather the sample will be pipetted directly into the sedimentation chamber. The SurePath system requires that a total 200µl of fluid is placed in the sedimentation chamber. For this part of the study, the 200 µl will comprise a combination of sample and collection fluid. The volumes for each dilution are given in Table 1 below. It is important that the collection fluid is added before the sample to avoid the latter drying out.
- Processing of the samples is then completed as per usual.

Table 1 – Schedule of dilutions for SurePath

Slide label	Expected Sample cellularity	Volume of sample to be added (µl)	Volume of diluting collection fluid (µl)
Accession no – D1	Original slide		
Accession no – D2	45k	150	50
Accession no – D3	30k	100	100
Accession no – D4	25k	83	117
Accession no – D5	22.5K	75	125
Accession no – D6	20k	67	133
Accession no – D7	15k	50	150
Accession no – D8	10k	33	167
Accession no – D9	5k	17	183
Accession no – D10	2.5K	9	191

ThinPrep

The production of dilutional slides for ThinPrep is less easy than for SurePath and will almost certainly require more trial and error. In order to produce poorly cellular slides the system will need to be offered super-dilute samples and the processor allowed to filter all or most of their volume. This effectively over-rides the error message which the system will display.

- The residual sample is currently stored in the original vial.

- Only cases where >15ml sample remains in the vial can be selected. A total of 12.945ml of sample is required to produce the suggested dilutions.

- The slides to be used in the T2000 machine must be appropriately labelled with the case accession number followed by the dilution number to ensure traceability. (If the methodology is successful it may be easier in subsequent runs to complete the same dilution for a block of cases rather than having differing dilutions on a single run).
- The residual sample is divided between 9 vials in the volumes given in Table 2 and topped up with PreservCyte to a total volume of 20ml. The vials can be recycled vials as minor contamination is not an issue.

- The vials are then offered to the T2000 in the usual way and stained/coverslipped prior to counting.

The following schedule (Table 2) is suggested as a trial:

Table 2 – Schedule of dilutions for ThinPrep

Slide label		Volume of sample to be added (ml)	Volume of PreservCyte (ml)
Accession no – D1	Original slide		
Accession no – D2	45k	5.0	15.0
Accession no – D3	30k	3.0	17.0
Accession no – D4	25k	1.5	18.5
Accession no – D5	22.5K	1.0	19.0
Accession no – D6	20k	0.75	19.25
Accession no – D7	15k	0.5	19.5
Accession no – D8	10k	0.25	19.75
Accession no – D9	5k	0.13	19.87
Accession no – D10	2.5K	0.07	19.93

Varying the proportion of normal/abnormal cells

This arm of the protocol requires that abnormal cases are mixed with known negatives in differing quantities to vary the number and relative proportion of abnormal cells. The protocols for these preparations again differ between the two LBC systems but for ease of production I would suggest that within LBC system all samples are handled identically.

At the recent Management Group meeting it was decided that to facilitate the circulation, 88 rather than 90 cases would be used and each of these would have 8 slides. The first slide will be the original preparation, hence an additional 7 slides will be produced.

SurePath

- 90 cases have already been earmarked for this arm of the study and the test tubes which have been topped up with 2ml of collection fluid are being held in reserve in the laboratory. Use numbers 1-88 only. Hold the other 2 cases in reserve in case there are problems. To make the preparations the residual material from 88 'negative' cases will also be needed. The original slides from these negative cases should be of average cellularity with no particularly recognisable background pattern i.e. not atrophic or especially inflammatory. LT will select these cases from current workload.
- Two 'stock' solutions should be made up intially.
- For 'stock 1'. Vortex test tube from case to homogenise sample. Pipette 500 µl of sample into clean test tube and add 4.5 ml of collection fliud. Total volume in test tube will then be 5.0ml.
- For 'stock 2'. Vortex test tube from case to homogenise sample. Pipette 50 µl of sample into clean test tube and add 5.0 ml of collection fliud. Total volume in test tube will then be 5.05ml.
- The slides to be used in the PrepStain machine must be appropriately labelled with the case accession number followed by the dilution number to ensure traceability.
- The PrepStain machine will be used as normal but there will be no samples in the sample test tube rack; rather the sample will be pipetted directly into the sedimentation chamber. The SurePath system requires that a total 200µl of fluid is placed in the sedimentation chamber. For this part of the study, the 200 µl will comprise a combination of 'stock' sample and a negative case. The volumes for each dilution are given in Table 1 below.
- Processing of the samples is then completed as per usual.

D1 Original slide		
	Negative sample vol in µl	Test sample vol in µl
		USE STOCK 1
D2	100	100
D3	150	50
D4	175	25
D5	190	10
		USE STOCK 2

D6	150	50
D7	175	25
D8	190	10

ThinPrep

- 90 cases have already been earmarked for this arm of the study and the vials are being held in reserve in the laboratory. Use numbers 1-88 only. Hold the other 2 cases in reserve in case there are problems. To make the preparations the residual material from 88 'negative' cases will also be needed. The original slides from these negative cases should be of average cellularity with no particularly recognisable background pattern (i.e. not atrophic or especially inflammatory) and each will require a minimum residual volume in the vial of at least 12ml and preferrably 15ml.

- The test vial should be centrifuged to obtain a cell button. The cell button should then be resuspended in 3ml of PreservCyte.

- The slides to be used in the T2000 machine must be appropriately labelled with the case accession number followed by the dilution number to ensure traceability.

- Decreasing volumes of test sample are then added to the residual volume in known negative cases as per following table:

D1 Original slide	
	Test sample vol in µl
D2	1,000
D3	500
D4	250
D5	100
D6	50
D7	25
D8	10

- The vials are then offered to the T2000 in the usual way and stained/coverslipped prior to counting.