Figure 1

Tissue collection
- Timing of sample: LH+7
- Mode of biopsy: pipelle sampler

Tissue fixation and processing
- Fixation: 10% neutral buffered formalin 24-48 hours, room temperature
- Processing: routine processing into paraffin wax, NOT xylene free

Tissue immunostaining and assessment
- Section thickness: 3µm, coated slides, ensure water bath is clean, stain within 1 month of cutting
- CD56 antibody: Leica Biosystems (NCL-CD56-504)
- Secondary/tertiary reagents: If hand staining then recommend use of Vectastain Elite ABC kit
- Detection system: DAB, light haemotoxylin counterstain
- Controls: negative (at minimum omission of primary antibody, preferably non-immune serum); known positive tissue with every staining run

Quantification
- Capture of images: 10 x400 images; start with luminal edge in view and image every 2nd field of view; if necessary can count 4 images deep from luminal edge.
- Counting: One operator/centre. Use Image J cell counter plug in to count total number of stromal cells and immunopositive uNK cells. Ensure that whole cells are being counted and not just fragments of DAB staining i.e. must see associated nucleus
- Number of cells counted: count at least 3800 stromal cells
- Calculation of results: express as % of total stromal cells
Figure 2

A

%uNK cells/stromal cells

Sample

Centre A
Centre B
Centre C

B

%uNK cells/stromal cells

Sample

Centre A
Centre B
Centre C
Figure 3

A

Collected in Centre A  Collected in Centre B  Collected in Centre C

Assessed in Centre A

Assessed in Centre B

Assessed in Centre C

B

A

Assessed in Centre B

Assessed in Centre C

B

Assessed in Centre C

Assessed in Centre B

Assessed in Centre A
Figure 4

A

%uNK cells/stromal cells

Expose kit

ABC vector kit

Routine Pathology Lab

Sample

6.0 7.0 8.0 9.0 10.0 11.0 12.0
464 743 1222 1848 2403 2946 3423 3845 4207 4437

%uNK cells/total stromal cells

Cumulative total number of stromal cells

Running average

Mean

B

Sample

C

+5%

-5%
Figure 5

A

B

Number of 40X fields from luminal edge
Figure 6

A

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<th>Sample</th>
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Legend:
- Black: Centre A
- Grey: Centre B
- Dark Grey: Centre C

B

Collected in Centre A
Assessed in Centre A

Collected in Centre B
Assessed in Centre B

Collected in Centre C
Assessed in Centre C