Cell Separation and Christmas Tree Staining for Sperm Cells		
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Cell Separation and Christmas Tree Staining for Sperm Cells

The following is a confirmatory test for the presence of spermatozoa and should be done only upon special request by a customer after consulting with a supervisor or manager. Epithelial cell lysis on a swab cutting or stain cutting can quickly eliminate any epithelial cells present. Any sperm cells then remaining can be viewed under the microscope after staining without epithelial cell masking. The nuclear material within the sperm cell head is stained red by the Kernechtrot Solution (Solution A). Any sperm tails present are stained green by the Picricindigocarmine Solution (Solution B).

1 Reagents:

- **Phosphate-buffered saline (PBS)**
- **Proteinase K**
- **Red Stain: Solution A (Kernechtrot Solution)**
- **Green Stain: Solution B (Picricindigocarmine Solution)**

2 Preparation of sample:

2.1 For swabs that yielded male DNA, the original smears in the kit should be stained first using the staining procedure in step 3 below. If sperm cells are seen, document their presence in LIMS and return the microscope slides to the kit box. If no sperm cells are seen in the original smears, or if no slides were previously prepared for the positive male DNA sample, then proceed with the following:

2.2 Cut approximately 1/3 of a swab or a 3x3mm cutting of a swatch of each sample into its appropriately labeled sample tube.

2.3 Add the following reagents to each sample and record their lot numbers in LIMS:

500µl PBS
1µl Proteinase K

2.4 Incubate samples at 56°C for 10 minutes using a shaking platform (1400rpm).

2.5 Centrifuge the samples briefly to spin down the condensation.

2.6 Place spin baskets in click fit tubes and label.

2.7 Using clean forceps, remove the substrate from each sample tube and place in spin basket inside a click fit tube. Add all remaining liquid to the spin basket as well.

2.8 Centrifuge the samples for 5 minutes at 6600 rpm.

2.9 Without disturbing the pellet, remove the spin basket and discard all but 50µl of the supernatant from the sample.

2.10 Resuspend pellet by pipetting up and down and/or vortexing.

2.11 With a permanent marker, circle an area approximately 10mm in diameter on a new microscope slide and pipette 10µl of sample into the circle.

3 Staining Procedure:

3.1 Fix cells to the slide by heating on hot plate (approximately 5 to10 seconds).

3.2 Cover sample area of slide with Solution A (red stain) and allow to sit for 10 minutes.

3.3 Wash away Solution A with deionized water.

3.4 Add Solution B (green stain) to the still-wet slide; allow to sit for 10 seconds.

3.5 Wash away Solution B with ethanol. Record lot numbers of both solutions in LIMS.

3.6 Place slide over hot plate to complete drying.

3.7 Examine the slide at 40X or 100X. If sperm cells are seen, document their presence in LIMS.

3.8 Place slide in slide holder, seal with evidence tape, and return with the evidence.