THE PRINCIPLES BEHIND PROCESSING LIQUID BASED CYTOLOGY using SUREPATH

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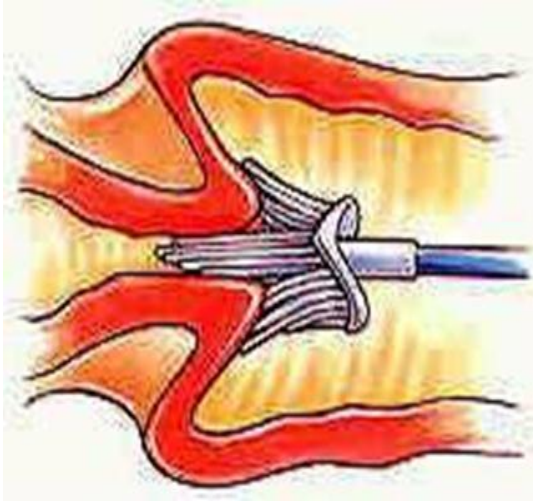
OVERVIEW

4 STEPS:

- SAMPLE COLLECTION
- SLIDE PREPARATION
- IMAGE CAPTURE
- AUTOMATED SCREENING

STEP 1:
SAMPLE COLLECTION

- Sample taken with plastic device



- The most commonly used sampling device is the cervibroom. A cytobrush can be used as well in specific clinical situations where particular attention to endocervical canal sampling is important.
- The sampling device goes into the larger of the two holes in the top of the vial. The head is then slipped off into the vial, using the internal plastic structure of the vial.
- SurePath preservative fluid is 24% ethanol based

STEP 2:
SLIDE PREPARATION

THE CELL ENRICHMENT PROCESS

Consists of:

a) Agitation

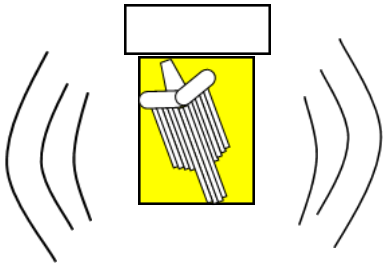
b) Density gradient centrifugation

c) Sedimentation

d) Image capture

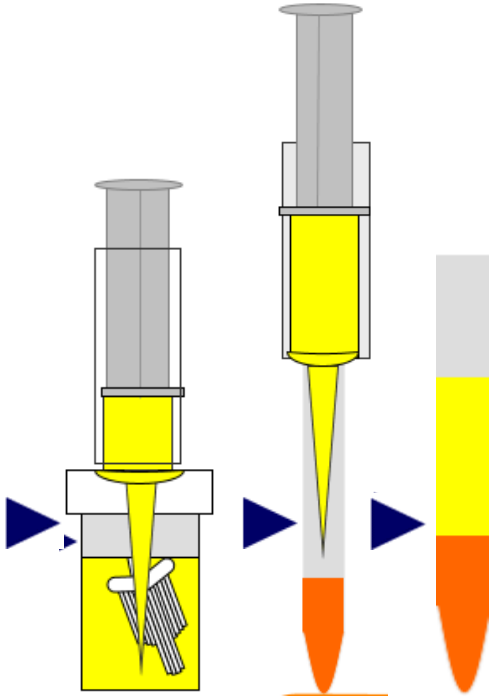
AGITATION

- Prepares the sample for the enrichment process
- Mixes the sample



AUTOMATED PIPETTING

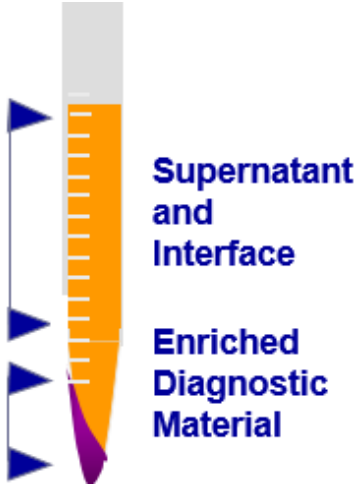
- PrepMate machine prepares samples for enrichment process
- Vial caps are pierced with syringe to avoid contamination
- Mixes and dispenses specimen onto density reagent in 12ml tube
- Density gradient formulated to separate cells from obscuring artefact like blood and mucus



DENSITY GRADIENT CENTRIFUGATION: FIRST CENTRIFUGATION

- Pulls cell solution through density gradient
- Density gradient separates cells from obscuring artefact like blood and mucus
- Supernatant is then decanted

First centrifugation
2 Minutes @ 200 x g



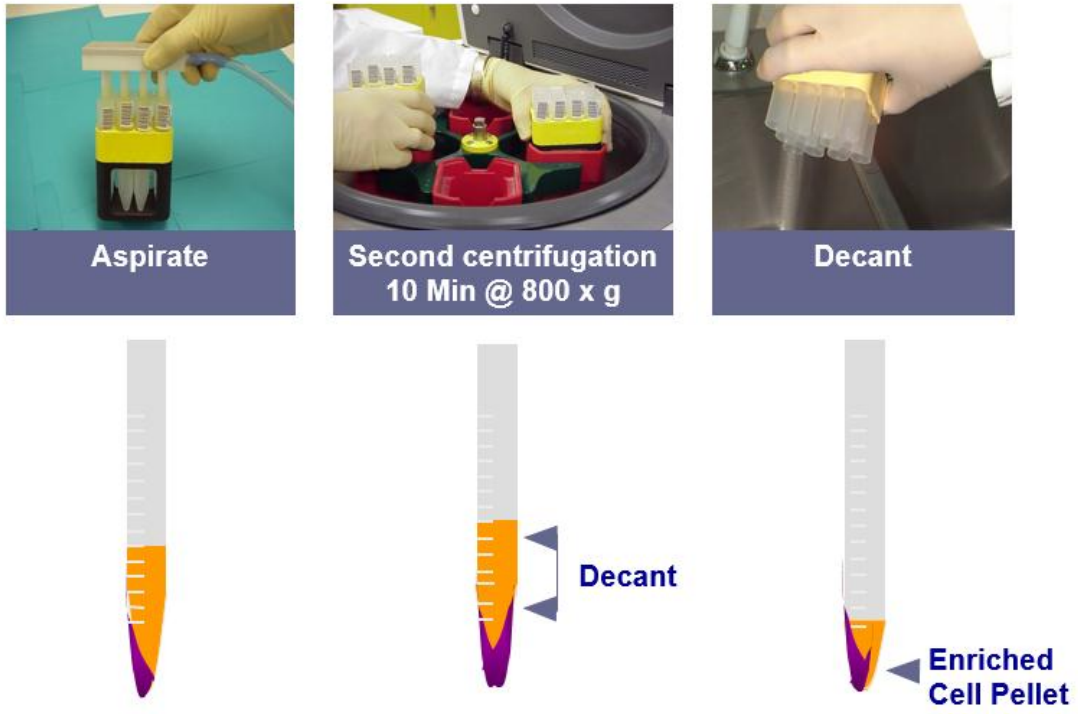
DENSITY GRADIENT CENTRIFUGATION

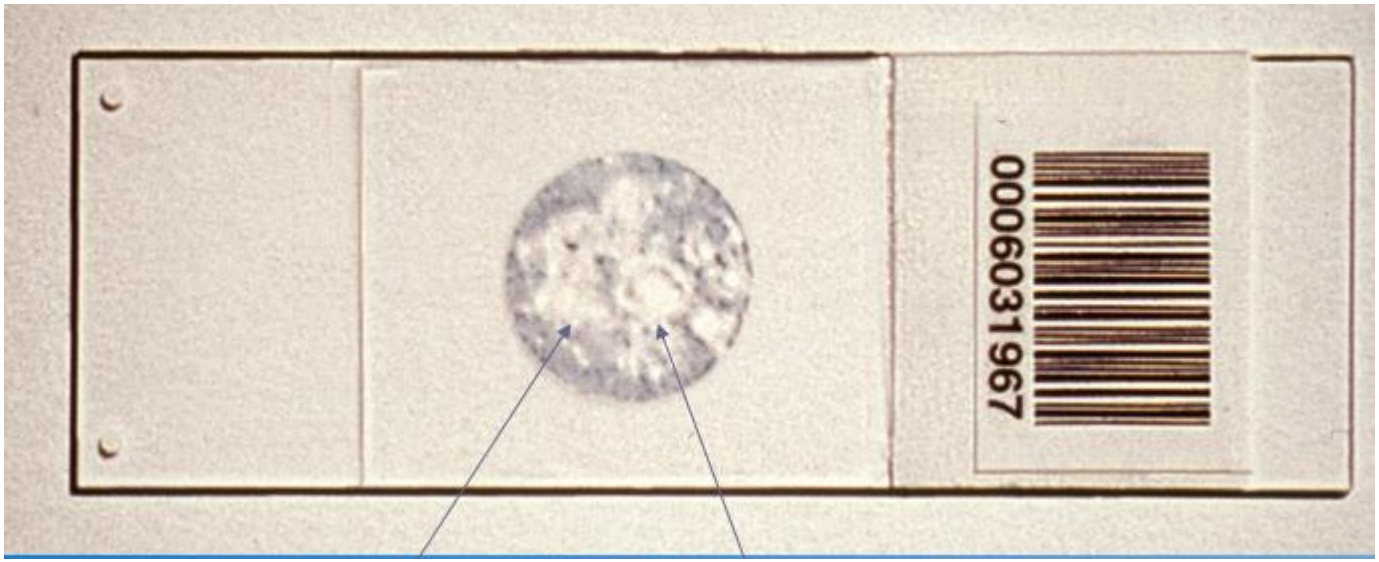
Density gradient centrifugation removes small particles such as aggregated proteins, disrupted membranes, microbial and red cell artefacts based on particle size.

- Non-diagnostic debris and excess inflammatory cells are largely removed from the sample.
- Air-drying artefact, obscuring, overlapping cellular material and debris are largely eliminated.
- The numbers of white blood cells are significantly reduced, allowing for easier visualization of epithelial cells, diagnostically relevant cells and infectious organisms.

DENSITY GRADIENT CENTRIFUGATION: SECOND CENTRIFUGATION

- 800g for 10 minutes
- Ensuing pellet consists primarily of epithelial cells

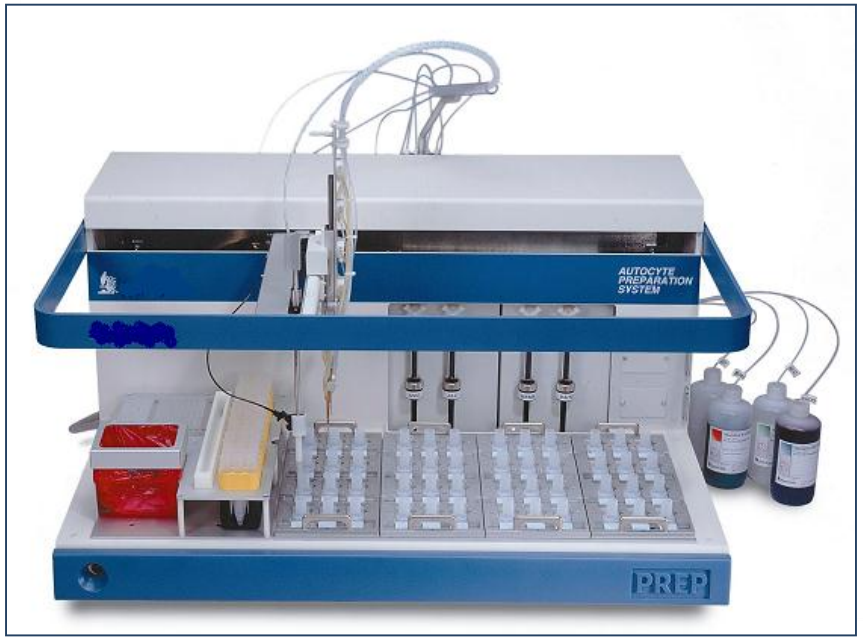




Arrows show clear areas on the SurePath slide where obscuring elements like mucus, blood and proteins have displaced epithelial cells because a density reagent has not been used.

SEDIMENTATION

- Occurs in PrepStain slide processor
- Enriched samples in centrifuge tubes are placed on the slide processor
- Cell pellet is re-suspended
- Robotic arm transfers sample from the tube to a settling chamber that sits on top of the glass slide
- Cells settle onto the slide by gravity



SEDIMENTATION

- Slides are coated with Poly-L-Lysine and air dried
- The beta helix structure of the Poly-L-Lysine molecule opens up to “grab” epithelial cells uniformly over the 13mm diameter preparation area
- Sedimentation time is 10 minutes
- Then drying time is 55-300 seconds—this allows the cells to dry on the slide and flatten. The longer the time, the less multilayered the preparation will be, resulting in easier screening

The Prepstain then carefully aliquots EA, Orange-G and haematoxylin stains onto the slides.

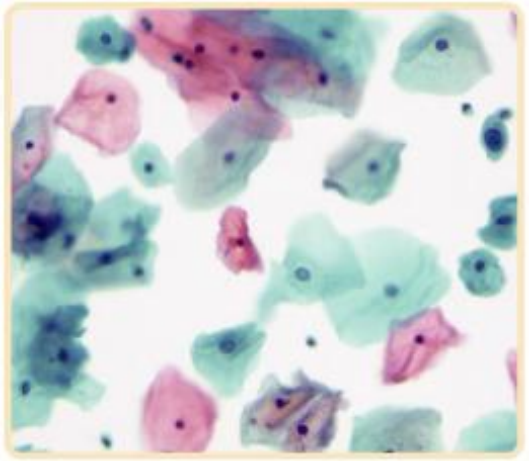
On completion a 13mm circle with approximately 75,000 cells is produced

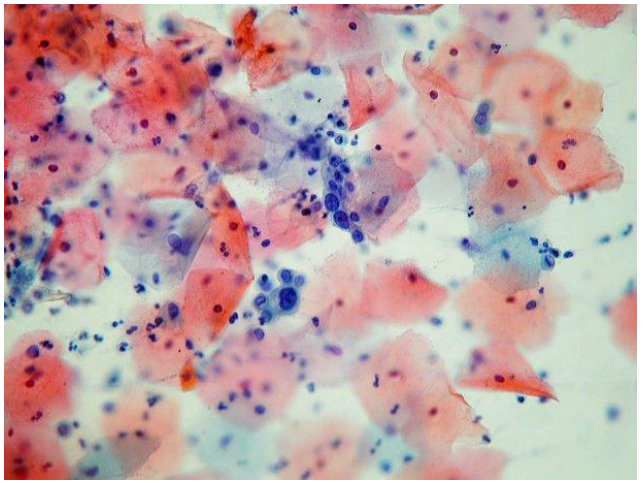
STAINING

- The Prepstain uses the basic Papanicolaou staining method to stain the slide preparation: a modified progressive Papanicolaou stain is used
- Pap staining differentiates cells on the slide
- SurePath uses a combined OG-6/EA solution
  - OG-6 (6 denotes the concentration of Phosphotungstic acid) stains keratin
  - EA contains 3 dyes:
    1. EOSIN Y stains superficial (mature) squamous cells, nucleoli, cilia, red blood cells
    2. LIGHT GREEN SF stains cytoplasm of other cells
    3. BISMARCK BROWN

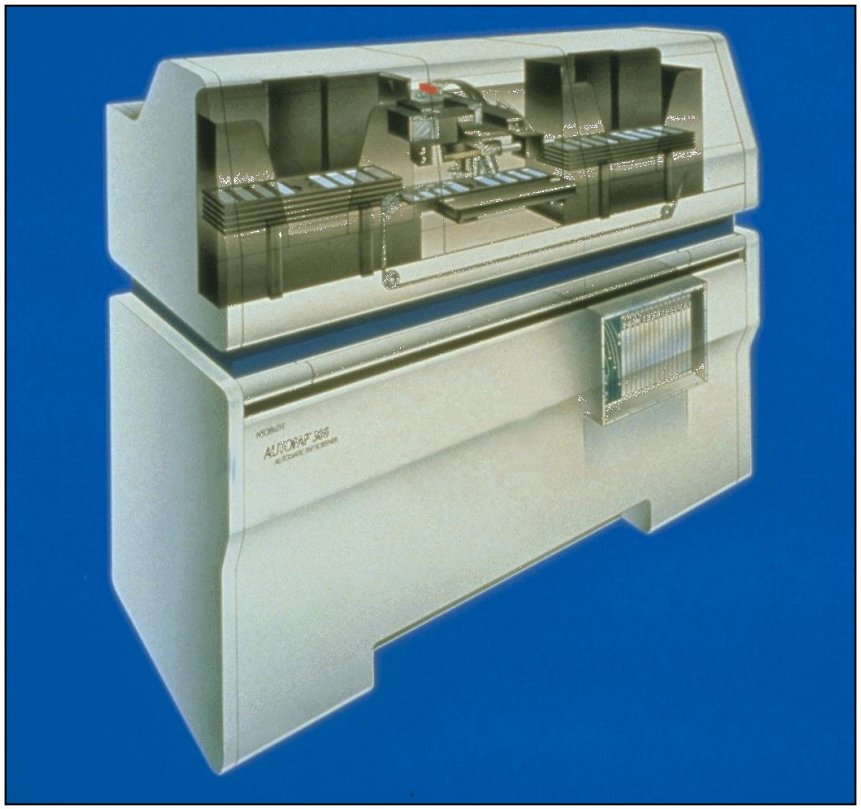
STAINING

- The resulting slide displays hues from the entire spectrum: red, orange, yellow, green, blue and violet
- Chromatin is well visualised, cells are transparent so even thick areas allow for examination of cells
- Haematoxylin stains nuclei and achieves a crisp blue to black effect





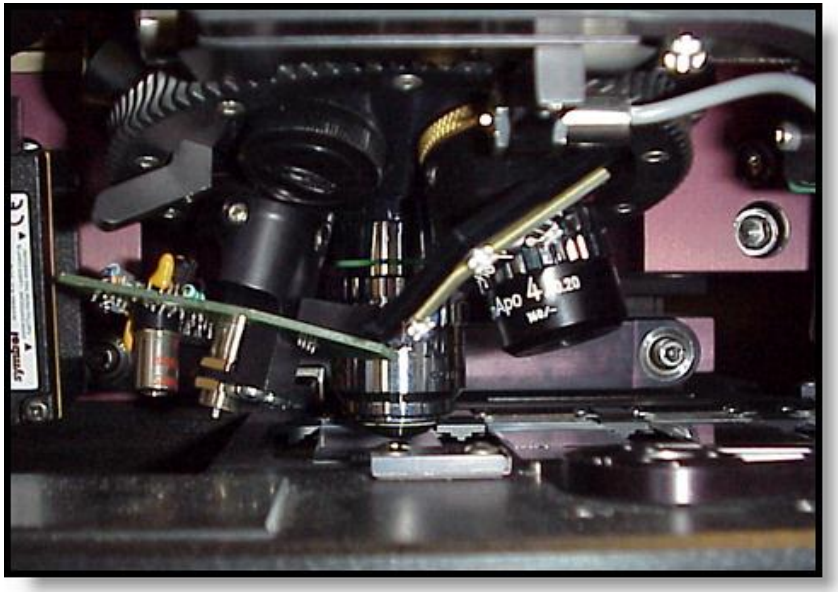
STEP 4:
FOCALPOINT SLIDE PROFILER



- Essentially a high speed video microscope
- Slides loaded on racks through input hopper

- Machine feeds trays through optical path, calibrating before and after each tray

FOCALPOINT PROFILER IMAGE CAPTURE



- Each slide undergoes a scan of 1000 fields of view (FoV) using a X4 objective.
- Up to 11 FoV are captured for examination using guided screening microscope
- Selected areas (FoV) are closely examined using a X20 objective
- 40 minutes are required to scan a tray of 8 slides

FOCALPOINT PROFILER
FIELD OF VIEW (FOV) COMPUTERS

- Slides are ranked and scored according to their likelihood of containing abnormal cells in batches of between 80 and 120 slides called a print set
- The first quintile ranking has the highest probability of containing abnormal cells
- The last quintile has the highest probability of being benign
- The presence or absence of endocervical cells is also reported

FOCALPOINT PROFILER CATEGORISING RESULTS

The guided screening microscope (slide wizard) is a workstation platform with a computer system linked to an automated microscope that allows a screener to advance the automated stage and examine each of the (up to) 11 FoV in slides that have come off the FocalPoint

All slides must be looked at by a screener qualified to work in an automated screening environment