

PRINCIPLES BEHIND
PROCESSING LIQUID BASED
CYTOLOGY
using
The THINPREP system

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AIMS of LBC PROCESSING

- INCREASE SPECIFICITY AND SENSITIVITY
- DECREASE THE MARGIN OF ERROR

PROBLEMS WITH CONVENTIONAL CYTOLOGY

- HIGH COST OF MANUAL SCREENING
- SIGNIFICANT FALSE NEGATIVE RATE BECAUSE:
 - SPECIMEN LARGELY POORLY FIXED
 - OBSCURING FACTORS e.g. BLOOD
 - HUMAN ERROR with DETECTION and INTERPRETATION



THE PLAN

- INTRODUCE COMPUTER IMAGE ANALYSIS

THE PROBLEM

- THE POOR QUALITY OF PAP SMEAR SLIDES MADE THIS IMPOSSIBLE

THE SOLUTION

- DEVELOP A BETTER WAY TO PREPARE SLIDES
- LIQUID-BASED CYTOLOGY IS DEVELOPED

THE PLAN

- CERVICAL SAMPLE COLLECTED
- PLACED INTO A LIQUID SUSPENSION
- CONTROLLED MEMBRANE TRANSFER SYSTEM
DEVELOPED TO SELECT A RANDOM SAMPLE OF
CELLS FOR MICROSCOPIC EVALUATION

THE PROCEDURE

- A SAMPLING DEVICE IS ROTATED 5x AROUND THE CERVICAL OS
- THEN RINSED VIGOROUSLY IN FIXATIVE

OBTAINING A SPECIMEN

OBTAIN...



RINSE...



TIGHTEN...



RECORD...



PLACE...



THE ADVANTAGES

- IMMEDIATE FIXATION
- ALL COLLECTED MATERIAL AVAILABLE FOR EVALUATION
- MULTIPLE SAMPLES CAN BE PREPARED
- CLEANER BACKGROUND—CELLS MORE VISIBLE
- THIN LAYER OF DISPERSED CELLS PLACED ON SLIDE
- UNSATISFACTORY RATE DECREASED
- SUITABLE FOR AUTOMATED ANALYSIS

THINPREP 5000 PROCESSOR



PROCEDURE STEPS in the PROCESSOR

- 1. CELL DISPERSION
- 2. CELL COLLECTION
- 3. CELL TRANSFER

STEP 1: CELL DISPERSION

- BREAKS UP: BLOOD, MUCUS, DEBRIS
- THOROUGHLY MIXES SAMPLE
- CREATES CURRENTS –
 - STRONG ENOUGH FOR DISPERSION WITH NO CELL CHANGE

STEP 2: CELL COLLECTION

- VACUUM CREATED WITHIN THE FILTER
- CELLS COLLECTED ON EXTERIOR SURFACE OF MEMBRANE
- RATE OF FLOW IS MONITORED TO PREVENT THE CELLULAR PRESENTATION BEING TOO SCANT OR TOO DENSE

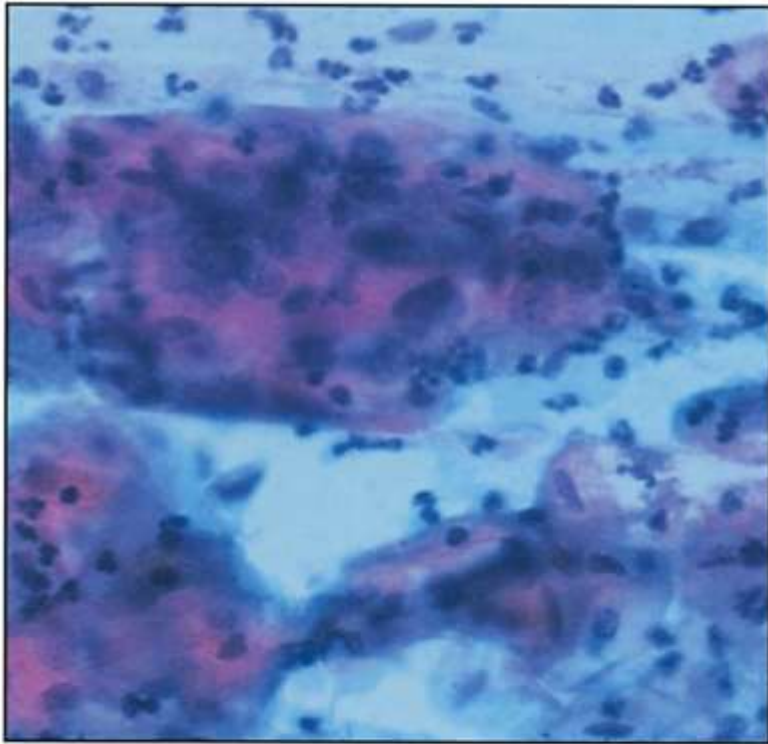


STEP 3: CELL TRANSFER

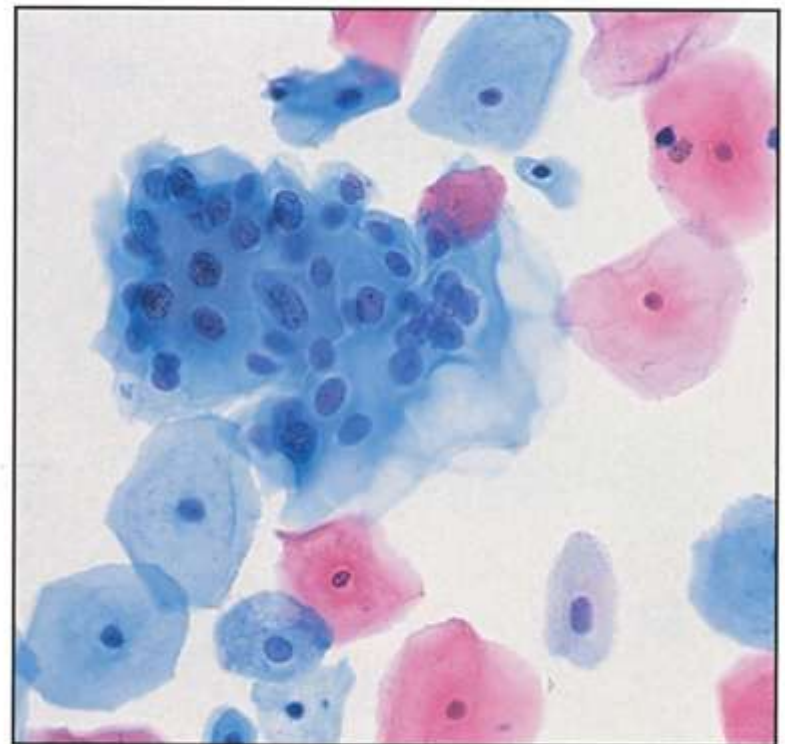
- CELLS COLLECTED ON MEMBRANE
- INVERTED
- FILTER PRESSED AGAINST MICROSCOPE SLIDE
- NATURAL ATTRACTION AND SLIGHT POSITIVE PRESSURE CAUSE CELLS TO ADHERE TO SLIDE.
- RESULTS IN AN EVEN DISTRIBUTION OF CELLS IN DEFINED AREA
- 20mm CIRCLE OF CELLS ON SLIDE
- SLIDE IS EJECTED INTO FIXATIVE BATH



THE OUTCOME



Conventional Pap smear slide



ThinPrep® Pap Test® slide

THE STAIN

- STOICHIOMETRIC STAIN –
 - THE DEGREE OF DARKNESS REFLECTS THE NUCLEAR DNA CONTENT
- RESULTS IN CRISP NUCLEAR DETAIL
- CLEAR CYTOPLASMIC STAINING
- TRANSPARENT CELLS

THE STAIN

- CONSISTS OF 5 MAIN SOLUTIONS:
 - NUCLEAR STAIN
 - RINSE SOLUTION
 - BLUEING SOLUTION
 - ORANGE G SOLUTION
 - EA SOLUTION

THE STAIN

- CONSISTS OF 2 PARTS:
 - FRONT END - AQUEOUS STAINING SOLUTIONS AND SOLVENTS:
 - THINPREP NUCLEAR STAIN, RINSE AND BLUEING
 - BACK END - ALCOHOLIC STAINS AND SOLVENTS:
 - THINPREP ORANGE G AND EA

THE STAIN

- SPECIMENS ARRIVE AT THE LABORATORY IN CYTOLYT FIXATIVE CONSISTS OF 95% ALCOHOL (METHANOL)

SO

- SPECIMENS REQUIRE THE ADDITION OF WATER SO THAT AQUEOUS STAINS CAN PENENTRATE THE CELLS. THIS IS CALLED HYDRATION

SPECIMENS ARE TAKEN THROUGH GRADED ALCOHOLS FROM 70% - 50% WATER BEFORE STAINING CAN BEGIN

THE STAIN – FRONT END

STEP 1

NUCLEAR STAINING:

- HAEMATOXYLIN IS USED (FROM THE LOGWOOD TREE):
 - THIS IS A DYE, NOT A STAIN
 - MUST BE OXIDISED TO HAEMATEIN
 - HAEMATEIN MUST COMBINE WITH A METAL SALT
(THINRPEP USES ALUMINUM-HAEMATEIN COMPLEX)
TO FORM A DYE MORDANT

THIS STAINS THE NUCLEUS BURGUNDY

AT pH 2.5

THE STAIN – FRONT END

STEP 2

RINSE SOLUTION:

- ACTIVE INGREDIENT IS:
 - A DETERGENT (pH 3.5)
 - FUNCTION:
REMOVAL OF LOOSELY BOUND DYE FROM THE CYTOPLASM

(ALUM-HEMATEIN BINDS WITH CYTOPLASMIC RNA)

THE STAIN- FRONT END

STEP 3

BLUEING SOLUTION:

- MAIN INGREDIENT : LITHIUM CARBONATE (pH>7.0)
- CAUSES NUCLEAR COLOUR CHANGE:
BURGUNDY TO BLUE-PURPLE

(DUE TO ALKALINE pH)

THE STAIN- BACK END

STEP 1

DEHYDRATION:

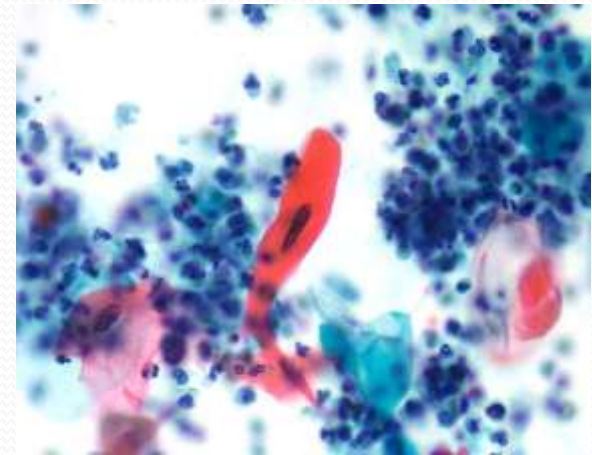
REQUIRED TO:

- SET UP CELLS FOR COUNTERSTAINING
- LOCK IN THE CHROMATIN STAINING

THE STAIN- BACK END

ORANGE G SOLUTION:

- STAINS KERATIN
- PROVIDES CLARITY TO CELL CLUSTERS



http://pathology2.jhu.edu/cyto_tutorial/Considerations/Images/Gu/1uripk2.jpg

THE STAIN- BACK END

STEP 2

EA SOLUTION – A MIX OF TWO DYES

STAINS CYTOPLASM:

- RED BY EOSIN
- GREEN-BLUE BY FAST GREEN

THE STAIN – BACK END

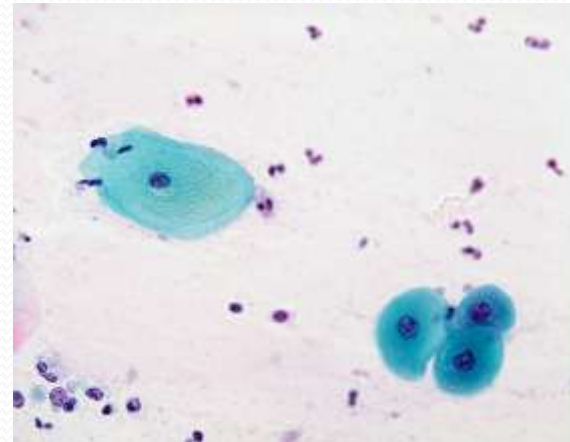
STEP 2

RED BY EOSIN



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GREEN-BLUE BY FAST GREEN



http://pathology2.jhu.edu/cyto_tutorial/Considerations/Images/FEMALE/N4oJCO.jpg

THE STAIN – BACK END

STEP 3

REMOVAL OF EXCESS COUNTERSTAINS:

- BY TWO BATHS OF 95% ALCOHOL
- TIMING IS FINITE— TOO LONG REMOVES BOUND DYE AS WELL

THE STAIN- BACK END

STEP 4

REMOVAL OF ALL WATER

- BY THREE BATHS OF 100% ALCOHOL
- CRITICAL TO DEHYDRATE COMPLETELY
- TIMING INFINITE

THE STAIN- BACK END

STEP 5

CLEARING AGENT

XYLENE PROVIDES :

- TRANSLUCENCY OF CELLS
- BRIDGE BETWEEN ALCOHOL AND MOUNTING MEDIA

THE STAIN- BACK END

STEP 6

MOUNTING MEDIA

PROVIDES :

- PHYSICAL PROTECTION OF THE SPECIMEN
- ANTIOXIDANT PROTECTION TO RETARD STAINING DEGRADATION
- PROPER REFRACTIVE INDEX TO GIVE UNDISTORTED VIEWING

THE THINPREP IMAGER

- SCANS ENTIRE SLIDE
- INDICATES THE 22 FIELDS OF VIEW CONTAINING THE MOST POTENTIALLY ABNORMAL CELLS

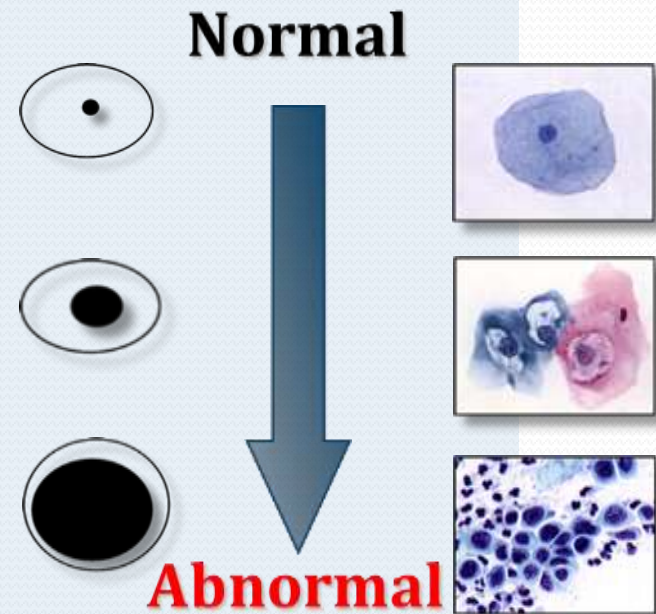
THE THINPREP IMAGER



SCREENING PRINCIPLE

Abnormal cells are known to have nuclei that are larger and contain extra copies of DNA

To identify cells for review the OCS Algorithms look for the “largest, darkest” objects



REVIEW SCOPE



Imaging:
Each slide is scanned in 90 sec.

Review Scope:
Presents the 22 fields of most interest determined by the Imager

IN SUMMARY

Collection



Physician rinses
Cervical Sample into
Vial

Preparation



ThinPrep® 5000
Processor



ThinPrep® Imaging
Stain

ThinPrep® Image Processor



Review Scope

Dual Screening