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LBC/HPV: Cervical cancer screening in the general Population-A Systematic Review

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Abstract

A monolayer slide preparation technique called liquid-based cytology (LBC) has surpassed traditional Pap smears due to enhanced fixation, a reduction in obscuring factors, and consistent cell transfer. Unlike conventional smears, in which the sample is smeared onto the glass slide and fixed separately, LBC involves collecting samples by fully submerging the sampling instrument into the company vial holding preservative solution. This preserves and fixes the cells concurrently. ThinPrep and SurePath are the two main liquid-based preparation techniques now in use. These two procedures have different cell harvesting principles, yet they create identical preparations. SurePath operates on the principle of density gradient sedimentation. A sample is vortexed and strained to break up mucus and big cell groupings, and then it is centrifuged using a density gradient. Staining with the PrepStain tool comes next. The SurePath method was approved by the FDA in 1999 and is used by Government Medical College and Hospital in Nagpur, India. Our organization uses the Rovers Cervex-Brush to gather cells from the transformation zone. The SurePath concept and the lab's fully automated cervicovaginal specimen processing method are covered in this chapter.

Keywords: Liquid-based cytology, SurePath, ThinPrep, Cervicovaginal, Cerves Brush Monolayer preparation, FDA, Harvesting

INTRODUCTION

For many years, pathologists have been trying to increase the "conventional Pap smear's" sensitivity and specificity. In the 1950s, cervical screening employed automated screening machines to reduce screeners' fatigue during this tedious and prone to error process. The initial few attempts, however, were unsuccessful because manually estimating the sizes of individual cells proved to be quite challenging, particularly in complex backgrounds .Consequently, pathologists emphasized the need for automated equipment that can provide representative monolayer cell samples on standardized slides that can be read by automated screening systems. Extensive research on this idea led to the creation of liquid-based cytology (LBC). LBC produced complete and consistent cell transfer, less obscuring factors, improved fixation, and shorter screening times in addition to monolayer preparation. As of right moment, ThinPrep and SurePath are the two major preparation techniques that are known. In 1996, the FDA approved ThinPrep as a cervicovaginal smear substitute in the United States. The usage of Autocyte Prep (sometimes referred to as SurePath or CytoRich Prep) was authorized in 1999.Though theoretically different, these two methods provide the same preparations.In 2008, the Pap test was replaced with liquid-based cytology as the cervical screening technology used by UK screening programs.[4] Pathologists have been trying to improve the "conventional Pap smear's" sensitivity and specificity for a long time. In an effort to lessen the fatigue screeners experienced during this tedious and prone to error task, automated screening equipment were launched for cervical screening in the 1950s. The initial attempts, however, were unsuccessful since it was exceedingly challenging to manually estimate the diameters of individual cells, particularly against a complicated background.As a result, pathologists stressed the development of automated machinery capable of producing representative monolayer cell samples on standardized slides that automated screening devices can read. Extensive investigation into this idea resulted in the development of liquid-based cytology (LBC). LBC produced full and consistent cell transfer, reduced obscuring factors, enhanced fixation, and shortened screening times in addition to monolayer preparation.

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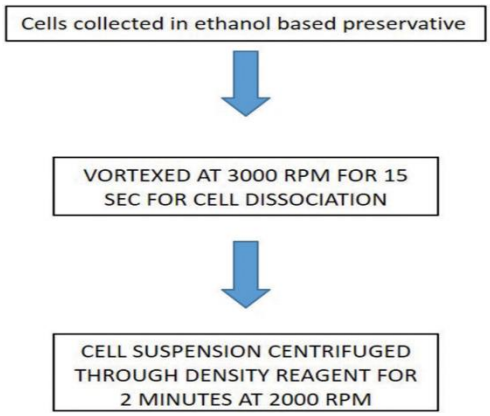
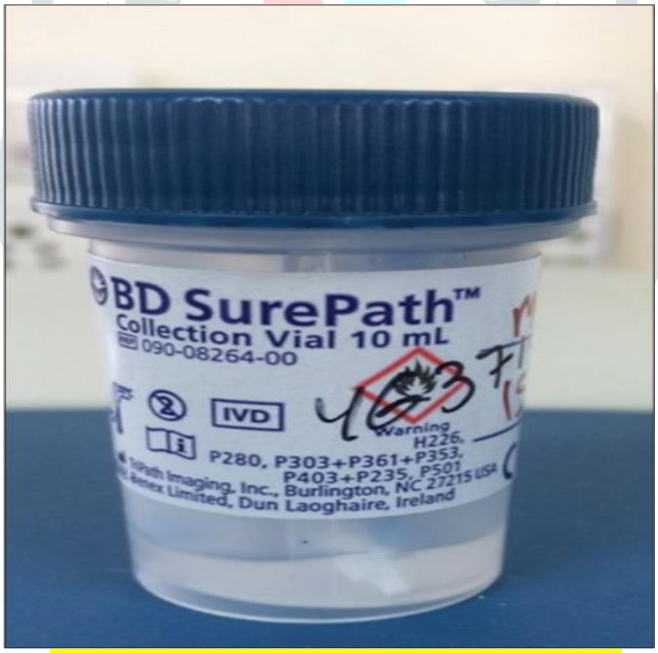


SUREPATH

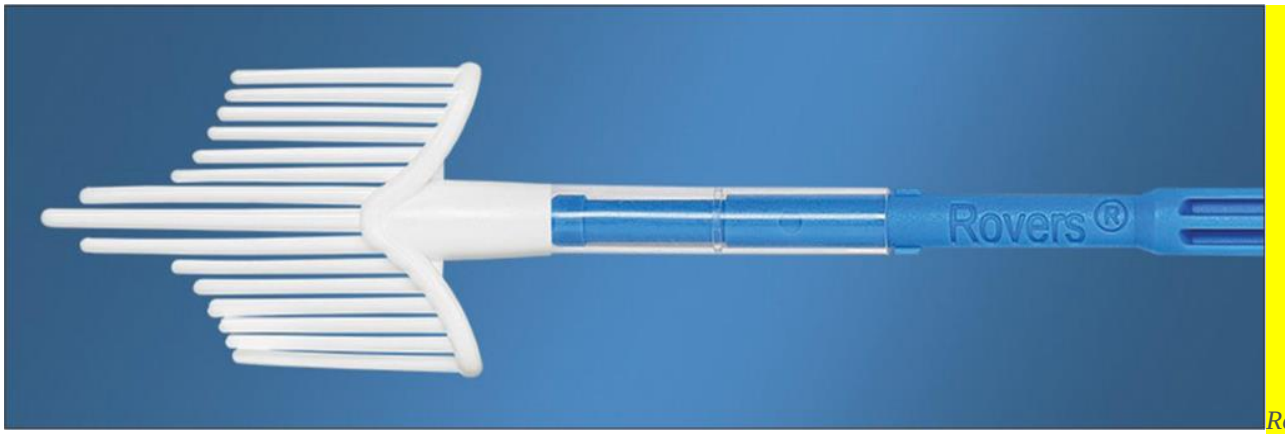
Rovers Cervex-Brush is the tool we use in our (GMCH) setup to obtain cervical sampleThe medical professional cuts the collecting device's tip and inserts it into the vial holding the ethanol-based preservative which simultaneously fixes and preserves the sample. BD Density gradient sedimentation is the foundation of SurePathTM's operation. In order to break up mucus and big cell groups, the cell or sample is vortexed and squeezed. Blood and debris are then removed using a density gradient centrifugation procedure. Cell pellets are resuspended and allowed to settle on a glass slide.

SurePath: The SurePath process involves vortexing, straining, layering onto a density gradient, and centrifugation. A centrifuge and a computer-controlled robotic pipette are essential instruments. The cells create a circle of 12.5 mm in diameter.

Thinprep: An apparatus, as well as special polycarbonate filters, are required for the ThinPrep process. After immersing the filter in the vial, the equipment rotates it to homogenize the sample. When a vacuum is applied to the filter, cells accumulate on its surface. The filter is then placed on a slide, transferring the cells to a 20 mm diameter circle.



Liquid-based cytology (SurePath) vial



Rovers Cervex-Brush

Tip of collection device (Cervex-Brush) in the vial containing ethanol

THINPREP

In this process, samples are collected in a vial with a filter and a methanol-based preservative (supplied by the manufacturer) . The vials are loaded one at a time into the semi-automated ThinPrep equipment. The filter inside the vial rotates mechanically, spreading cells, mucous, blood, and debris. This cell suspension passes through a neutral polycarbonate filter. The flow of this solution is regularly checked to ensure an adequate number of cells. Cells trapped on the filter surface are automatically transferred to a glass slide and instantaneously fixed. The TP-2000 processor is a semi-automated device that processes one sample at a time. A newer model, the TP-5000 is a fully automated bench-top equipment that processes specimens in batches of 20. A single vial can be used to make multiple different formulations. The clinician's sample is fixed in CytoLyt solution (a methanol-based fixative with hemolytic and mucolytic properties). The material is tagged and delivered to the cytology lab. The company provides microscopic slides, which are marked with a 20 mm diameter circle.The vial and a labeled slide are placed into the ThinPrep processor. Preparatory steps include specimen dispersion, collection, transfer, and staining.

Thin Prep PAP Test

TP-2000 processor

TP-5000 Processor

Dispersion

A disposable cylinder with a polycarbonate filter on one end is inserted into the vial. The filter's pore size is 8 µm. The instrument is rotated to create a current that disaggregates blood, mucus, and other debris, breaks up large cell clusters, mixes, and homogenizes the cell suspension.

Collection

A mild vacuum is provided to the cylinder, aspirating the cell suspension via the filter . Most fragmented red blood cells and detritus pass through, while diagnostic cells adhere to the filter's external surface. The instrument measures cell density across the filter, and the flow rate lowers when cells are equally distributed with minimal overlap.

Transfer

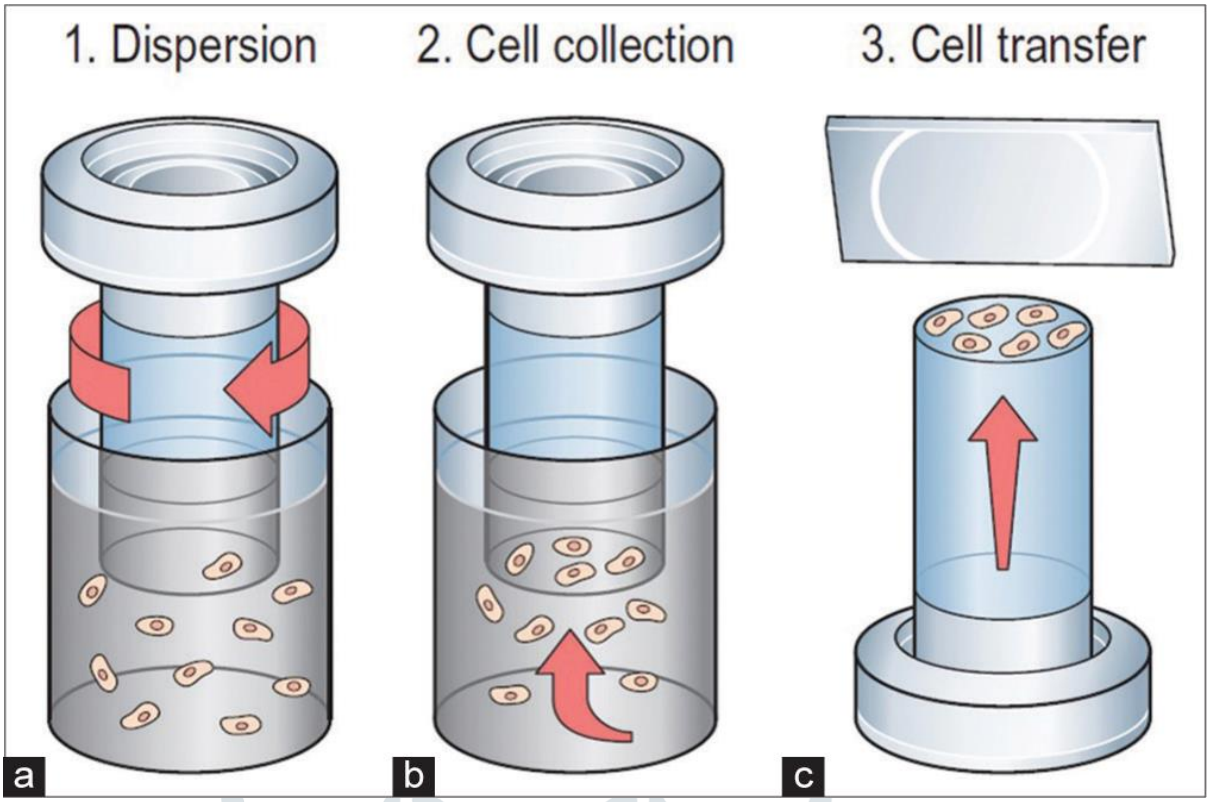
The cylinder is removed from the specimen, inverted 180°, softly pressed against a positively charged slide, and the cells are transferred to the glass slide using slight positive pressure . The outcome is a 20 mm circular smear with well distributed cells and minimum overlap.

Staining

Papanicolaou staining is done either manually or using an automatic stainer. The staining procedure takes 30 minutes. The Papanicolaou stain on fixed samples is the finest alternative for assessing the fine details of cell structure.

Residual LBP specimen

The residual specimens for SurePath and ThinPrep have a storage life of three weeks and three months, respectively, at room temperature. A leftover specimen might be employed in immunochemistry, molecular assays, or as a cell block.



Comparison to conventional smear

Liquid-based cytology increases specimen adequacy. The most notable changes observed with the ThinPrep approach include: Selective removal of extracellular material, which keeps it from obscuring cells but may result in the loss of diagnostic evidence. Selective loss of tiny cells. Fragmentation of big sheets and papillae Lack of smearing artifacts observed on traditional smears, which typically aids in the diagnosis but also eliminates the chance to observe distinctive smearing artifacts such chromatin smearing in small-cell carcinoma.Early trials, as examined on SurePath and ThinPrep, demonstrated increased detection of high-grade cervical intraepithelial neoplasia (CIN2 and CIN3),. However, subsequent meta-analyses and prospective randomized trials have not been able to show a statistically significant difference in the detection of CIN2 and CIN3 between conventional smears and liquid cytology

SPECIMEN HANDLING AND CELL ENRICHMENT

The cellular composition of the sample is enriched and the obtained cervicovaginal material is randomized during this stage of the BD SurePathTM slide preparation procedure. The sample first goes through a number of manual phases in this procedure, which include:

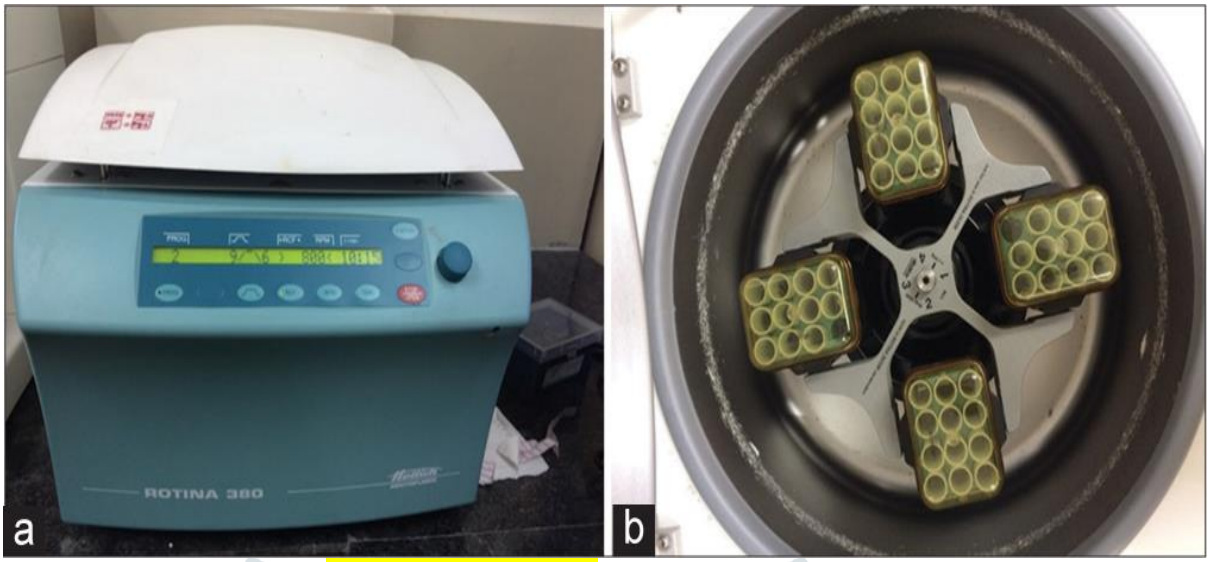
Sample randomization: The vortex machine vortexes received vials for 30 seconds at 3000 rpm. In addition to allowing the cells to separate from one another, this step releases the cell clusters from the specimen collection equipment.

Mixing and layering: PrepMate is used to automatically carry out this process. It is an automatic PrepStain system add-on. A centrifuge tube with 4 ml of density reagent, a sample vial, and a plunger are all inserted into the PrepMate specimen rack. Preservative sodium azide is added to a polysaccharide solution to create the PrepMate density reagent. The initial enrichment step, which involves mixing and dispensing the material over the density reagent, is automated by the PrepMate. Twelve samples can be kept in one specimen rack at once. The system has a total of three specimen racks, meaning that 48 samples can be run at once.

The specimen is mixed, taken out of the preservation vial, and layered onto the density reagent in the centrifuge tube using the PrepMate. One to twelve specimens are processed in a cycle by the automated PrepMate procedure. The layering procedure involves combining 8 milliliters of sample and 4 milliliters of density reagent in a centrifuge tube that is part of the rack.After combining and stacking the samples, the racks are taken out of the PrepMate. The sample and density reagent-filled centrifuge tubes are moved to the centrifuge apparatus . After two minutes of centrifugation in the tubes, the supernatant is disposed of. The removal of small particles and trash trapped at the interface between the density reagent and the supernatant preservative fluid enriches the clinical materials in the sample. After another 10-minute centrifugation, the tubes are turned 180 degrees to discard the supernatant. In this process, the diagnostic cellular material (pellet) is concentrated at the tube's bottom.After 30 seconds of vortexing, the pellet is put on the PrepStain device to be processed further.

Vortex device

PrepMate device.



Centrifugation machine

SPECIMEN PROCESSING WITH THE PREPSTAIN SYSTEM

For the thin layer preparation of cytologic material on a BD SurePathTM pre-coat slide, the PrepStain system automates the staining and slide preparation processes. The PrepStain system consists of the following components:

1- PrepStain settling chamber holds reconstituted cell material until it settles onto the covered microscope slide.

2-Computer workstation: The PrepStain instrument connects to a DOS computer system. The software that runs the sample processor is started by typing command at the DOS line and then manipulated through several menus.

3-The automated sample transfer and staining procedures are carried out by the PrepStain device .

4-Robotic sample processor: The PrepStain system's completely automated base instrument. It's a microprocessor-based liquid handling system with components managed by system software kept on a personal computer's hard drive. This device transfers aliquots of cell suspensions to settling chambers placed on BD SurePathTM pre-coated slides after resuspending the pelleted cell samples in buffered deionized water (DI). The processor uses the Papanicolaou method to stain the slides by performing a series of washings and staining procedures following a predetermined incubation period. The following is a list of the processor's main parts.

(a)-Syringe pump: This is a microprocessor-controlled syringe that attaches to a reagent container and quad tubing via tubing. It has a pump and a two-way valve. Four syringe pumps on the PrepStain device allow for very accurate, variable-speed aspiration and dispensing of the preprogrammed volume of reagents and samples.

(b)-The robotic arm's X/Y/Z movement mechanism allows it to move in the following directions: X (left-right), Y (forward and backward), and Z (up and down). It has an adjustable Z-rod .

(c)-The Disposable Tip (DiTi) assembly is utilized for sample pipetting and aspiration. Each sample is mixed, aspirated, and transferred to the settling chamber using a new pipetting tip from the PrepStain device. Z-rod mounting is used for the DiTi.

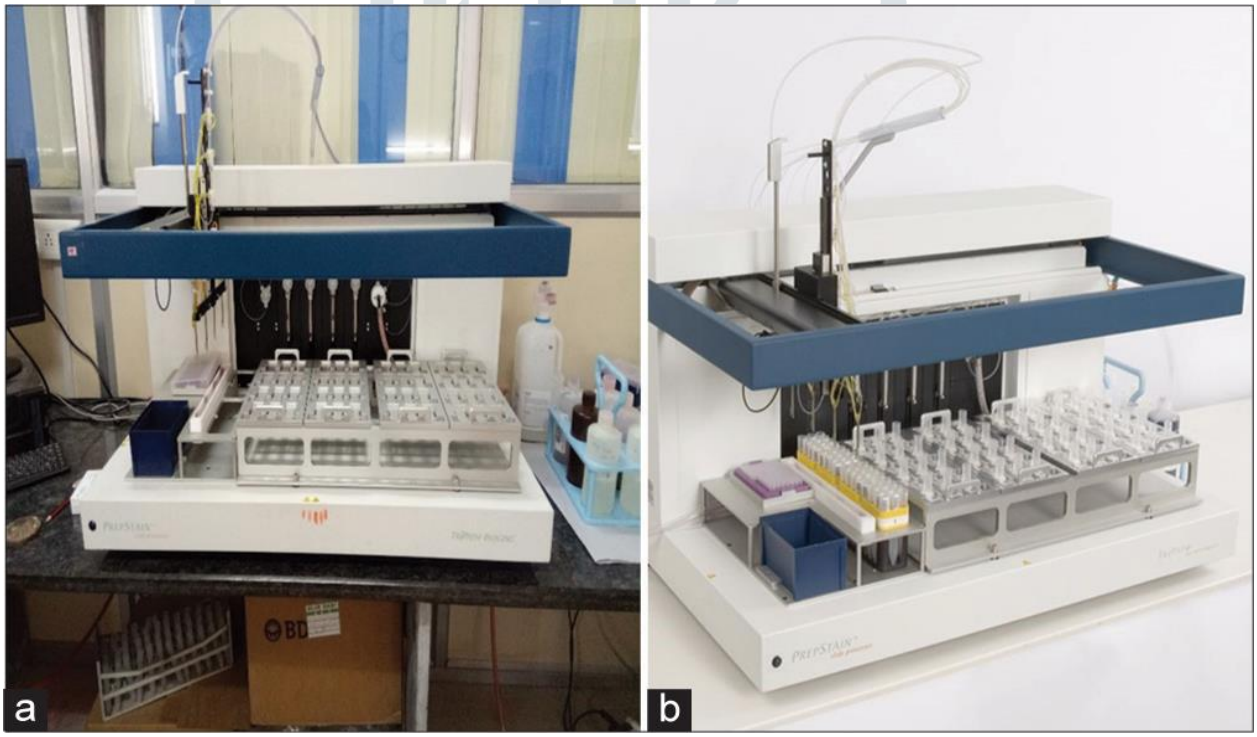
(d)-Quad arm : The PrepStain's robotic arm is equipped with a system of pipettes, tubing, and manifolds.

(e)- Instrument. The quad arm's four pipette bundles are positioned into the four settling chambers in the slide rack by the robotic arm. Four dispensing tips and one big vacuum tip are included in each pipette bundle. The settling chamber's supernatant fluid is emptied by the vacuum tip. One of the four reagents for the stain and rinse sequence can then be applied to the chambers using the four dispensing tips .

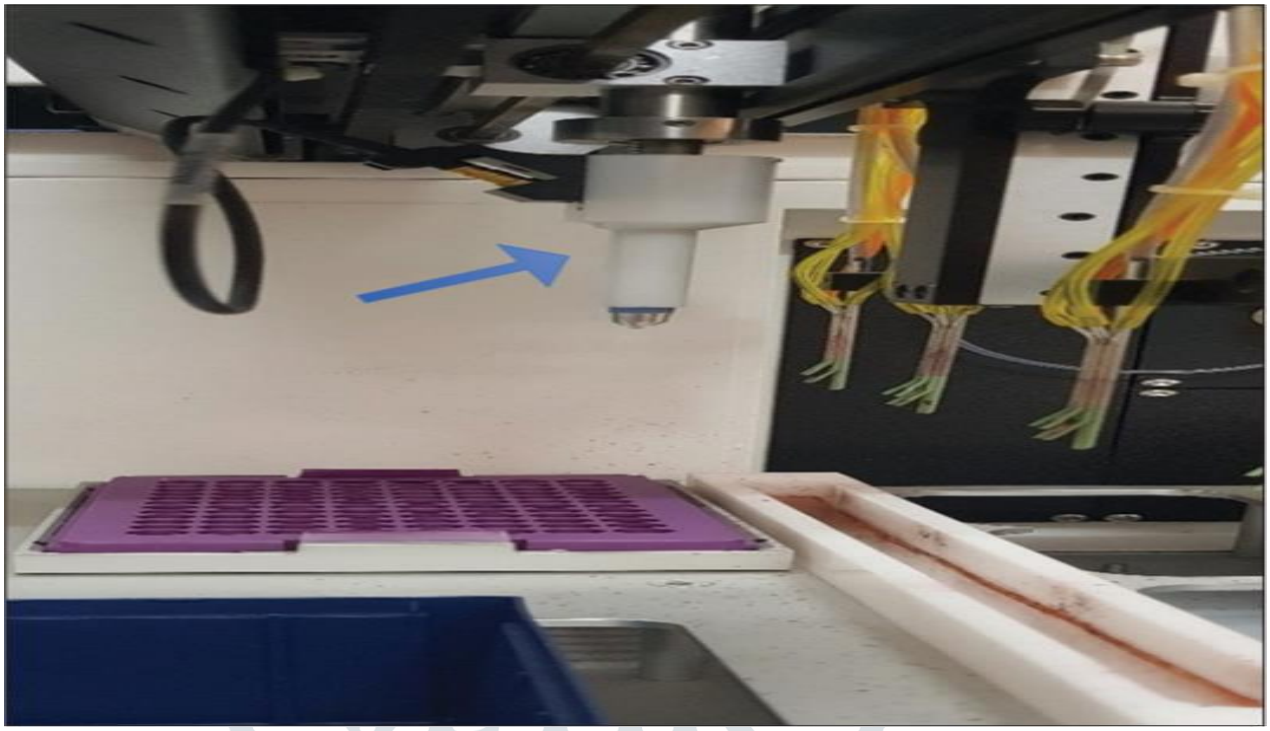
(f)-The waste station is located on the instrument's left side. Excess liquids are poured into the waste trough during system tubing priming or cleaning, and then they drain into a waste container for simple and secure disposal. DiTi is likewise disposed of in the waste receptacle after usage.

(g)-Work platform and slide racks : The work platform mounts to the right of the garbage station and accommodates the slide racks. Glass slide positions are arranged in an array of four rows by three columns on each slide rack. Slide racks and the work platform are numbered 1-4 and pinned, so that each slide rack can only fit into the designated slot. On the rack, each slide is positioned beneath a settling chamber. When the chamber is full with liquid, the settling chamber seal and the slide combine to create a barrier that stops leaks. In the settling chamber, the cells are sedimented onto the slide and stained. The settling chamber is taken out and disposed of after staining. The cover and slide have been cleaned

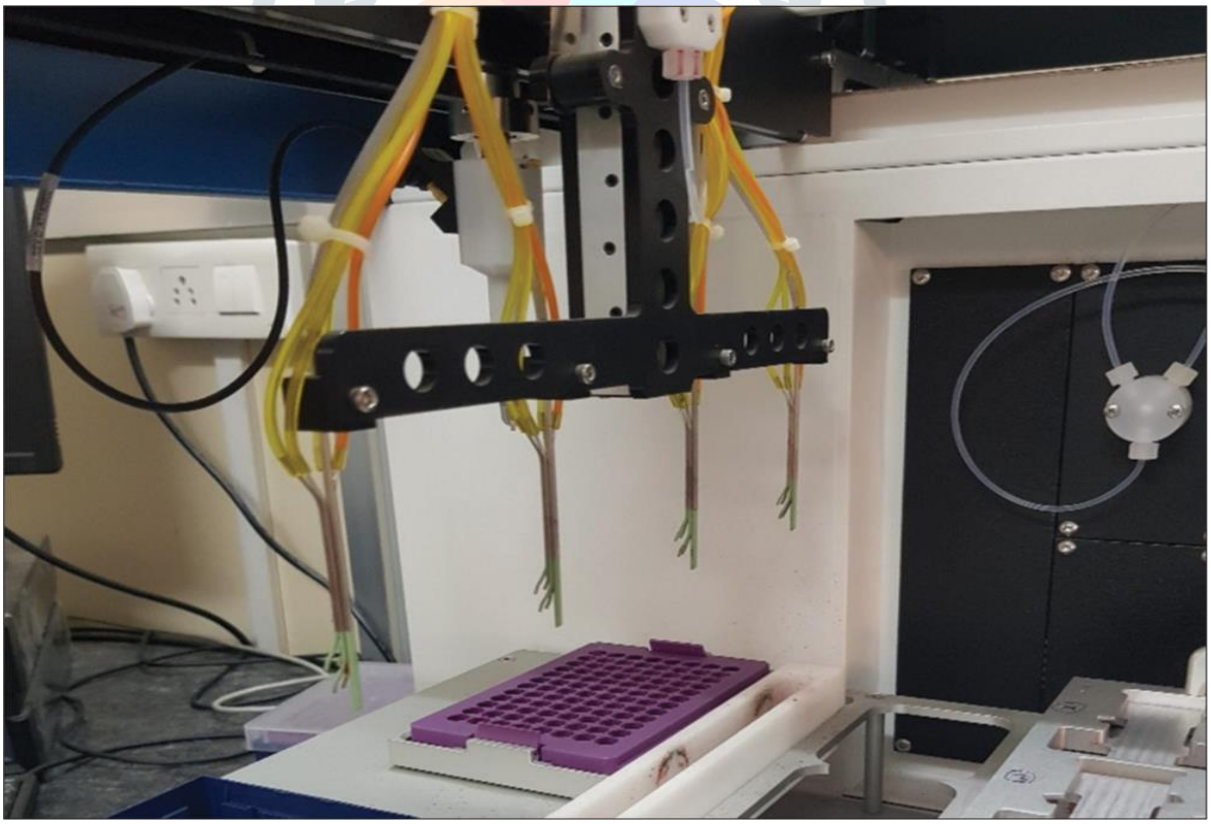
PrepStain settling chambers.



PrepStain device

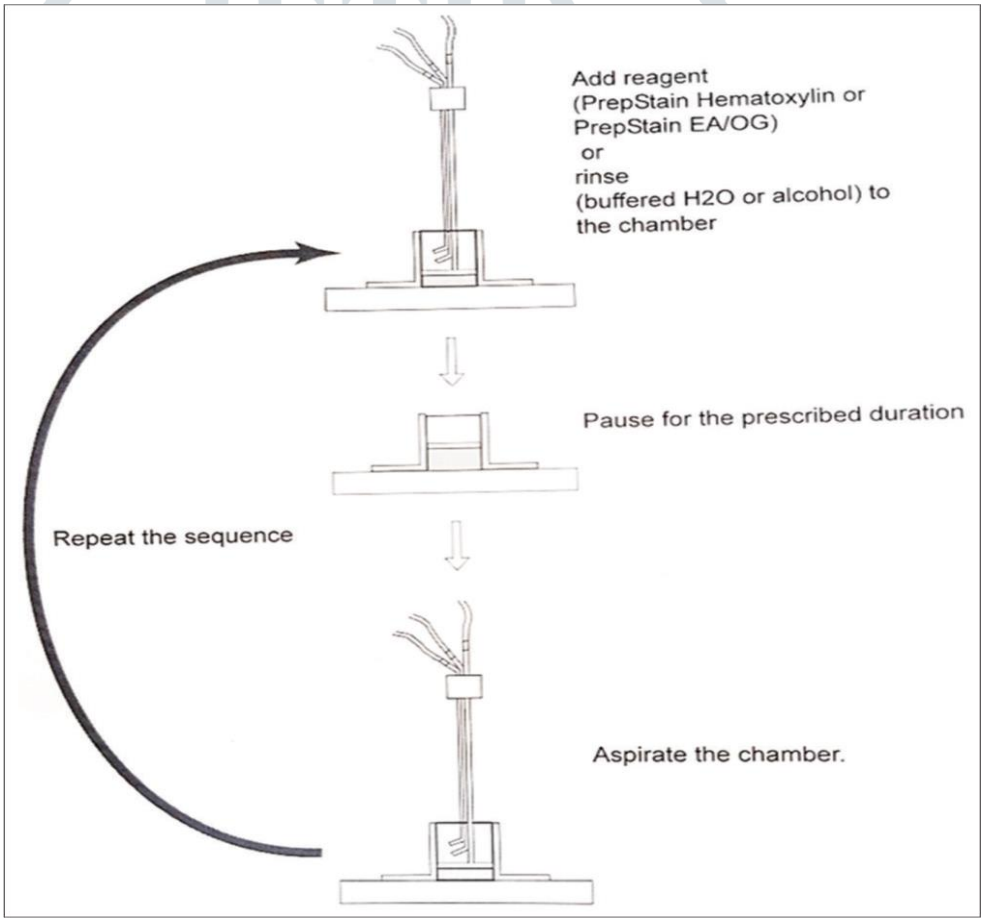


Z-rod (arrow) on the robotic arm

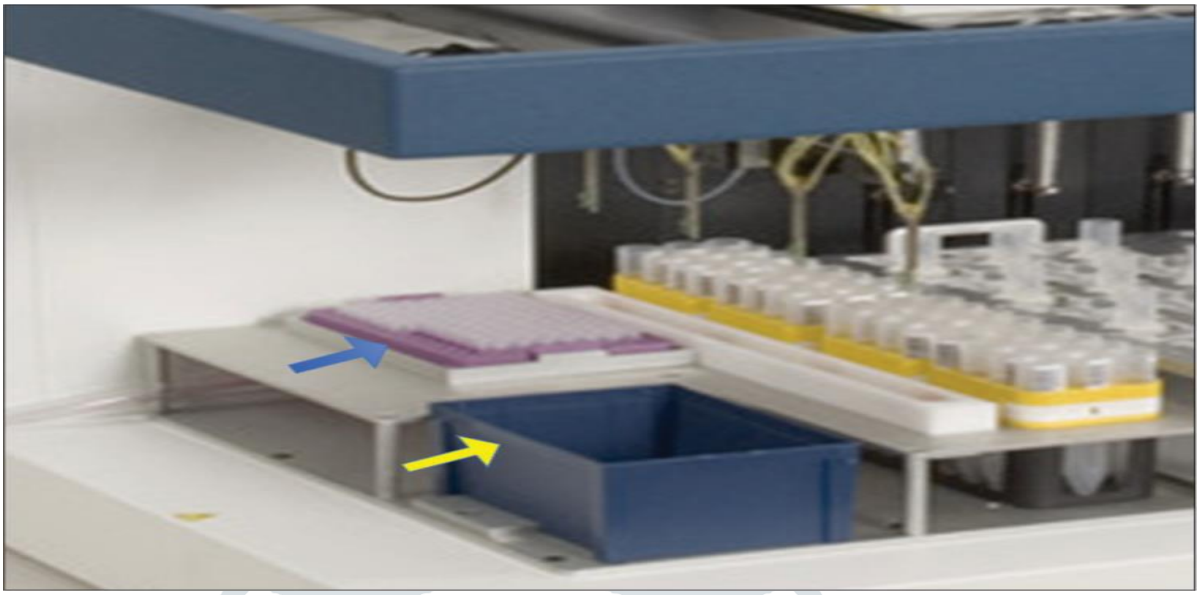


Quad arm with four pipette bundles

Slide racks and work platform



Stain and rinse cycle



Waste station with pipetting tips (blue arrow) and waste container (yellow arrow)

STAINING WITH PREPSTAIN INSTRUMENT

1. Slides labeled with the corresponding vial numbers are placed on the PrepStain machine's staining rack.
2. Before directing the staining process, the PrepStain apparatus is primed.
3. First, the cell pellet is mixed with 1000 µl of buffered DI water.
4. After that, the device takes up a DiTi and flushes the resultant solution eight times in and out of the disposable pipette tip.
5. After that, the device injects 200 µl of the sample into the settling chamber by aspirating it out of the centrifuge tube. It then cleans the tip with 600 µl of buffered DI water. You can either trash the tube and the remaining samples or keep them for follow-up examination
6. At least ten minutes are given for the sample to settle onto the slide. In this period, a thin layer of cells forms as a result of cells adhering to the PrepStain slide covering.
7. The instrument applies a 600 µl alcohol wash to the sample and removes any leftover fluids.
8. Allow the sample to dry for around 60 seconds.
9. The automated processing ends with stain and rinse cycles.
10. The PrepStain technique creates a homogeneous layer of stained cells in a 13 mm diameter circle . The sample layer consists of individual cells or tiny groups.
11. Staining racks are taken down once the staining is finished

BD SurePath slide with monolayered 13 mm diameter circle smear

Declaration of competing interest

The authors state that they have no known competing financial interests or personal ties that could have seemed to affect the work described in this study.

References

1-Liptak, Peter; Barnetson, Robert James (2012-02-14). "Liquid-based cervical cytology in the United Kingdom and South Africa". Continuing Medical Education. **30** (2): 45–48. ISSN 2078-5143.

2-Wilbur, David C.; Bibbo, Marluce (2008), "Automation in Cervical Cytology", Comprehensive Cytopathology, Elsevier, pp. 1021–1042, doi:10.1016/b978-141604208-2.10034-x, ISBN 978-1-4160-4208-2, retrieved 2020-07-27

3- Gibb, Randall K; Martens, Mark G (2011). "The Impact of Liquid-Based Cytology in Decreasing the Incidence of Cervical Cancer". Reviews in Obstetrics and Gynecology. **4** (Suppl 1): S2–S11. ISSN 1941-2797. PMC 3101960. PMID 21617785.

4-Liquid Based Cytology (LBC), NHS cervical screening programme (accessed 28/07/2014)

5-Michael CW, McConnel J, Pecott J, Afify AM, Al-Khafaji B (2001). "Comparison of ThinPrep and TriPath PREP liquid-based preparations in nongynecologic specimens: a pilot study". Diagn Cytopathol. **25** (3): 177–84. doi:10.1002/dc.2033. hdl:2027.42/35308. PMID 11536442. S2CID 7717237.

6-Lee KR, Ashfaq R, Birdsong GG, Corkill ME, McIntosh KM, Inhorn SL (1997). "Comparison of conventional Papanicolaou smears and a fluid-based, thin-layer system for cervical cancer screening". Obstet Gynecol. **90** (2): 278–84. doi:10.1016/S0029-7844(97)00228-7. PMID 9241308.

7-Bishop JW, Bigner SH, Colgan TJ, Husain M, Howell LP, McIntosh KM; et al. (1998). "Multicenter masked evaluation of AutoCyte PREP thin layers with matched conventional smears. Including initial biopsy results". Acta Cytol. **42** (1): 189–97. doi:10.1159/000331545. PMID 9479339. S2CID 3362795.

8- Siebers AG, Klinkhamer PJ, Grefte JM, Massuger LF, Vedder JE, Beijers-Broos A; et al. (2009). "Comparison of liquid-based cytology with conventional cytology for detection of cervical cancer precursors: a randomized controlled trial". JAMA. **302** (16): 1757–64. doi:10.1001/jama.2009.1569. PMID 19861667. S2CID 71747028.

9-Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J (2008). "Liquid compared with conventional cervical cytology: a systematic review and meta-analysis". Obstet Gynecol. **111** (1): 167–77. doi:10.1097/01.AOG.0000296488.85807.b3. PMID 18165406. S2CID 4918809. Review in: "Is liquid-based cytology better than Pap tests for CIN 2?". The Journal of Family Practice. **57** (4): preceding 220. 2008. PMID 18399015.

10- Cibas, Edmund S.; Ducatman, Barbara S. (2021). Cytology : diagnostic principles and clinical correlates. phia, PA. ISBN 978-0-323-63637-7. OCLC 1138033641.

11-Denton KJ. Liquid based cytology for cervical screening In: **Underwood J**, **Pignatelli M**, eds. In Recent Advances in Histopathology. Vol Vol 22. London: The Royal Society of Medicine Press Limited; 2007. p. :127-44.[Google Scholar]

12-Birdsong GG. Automated screening of cervical cytology specimens. *Hum Pathol*. 1996;27:468-81.[CrossRef] [Google Scholar]

13-Operator's Manual-prepstain Slide Processor- 780-13000-00 Rev D. United States: Scribd Inc.; 2014.