

## Methods of Detecting Cervical Cancer

*Krishnakumar Duraisamy, K.S. Jaganathan and Jagathesh Chandra Bose*

Shantha Biotechnics, Hyderabad, India

**Abstract:** Cervical cancer is the second most common cancer in women worldwide, with more than half a million new cases diagnosed in 2010. The disease disproportionately affects the poorest regions—more than 80% of cases are found in developing nations, mainly in Latin America, sub-Saharan Africa and the Indian subcontinent. Cervical cancer is an important cause of early loss of life as it affects relatively young women. Important advances have taken place in the diagnosis and treatment of this cancer in recent years. Surgery or chemo radiotherapy can cure 80-95% of women with early stage disease (stages I and II) and 60% with stage III disease. An ideal screening test is one that is minimally invasive, easy to perform, acceptable to the subject, cost-effective and efficacious in diagnosing the disease process in its preinvasive or early invasive state when the disease process is more easily treatable and curable. A variety of screening tests have therefore been developed in an attempt to overcome the innate limitations of conventional cytology. Screening techniques for cervical cancer include Conventional exfoliative cervicovaginal cytology i.e. the cervical (Pap) smear, Fluid sampling techniques with automated thin layer preparation (liquid based cytology), Automated cervical screening techniques, Neuromedical systems, HPV testing, Polar probe, Laser induced fluorescence, Visual inspection of cervix after applying Lugol's iodine (VILI) or acetic acid (VIA), Speculoscopy, Cervicography. The screening strategies mentioned above though applicable to the developed world may not be cost effective enough for widespread application in the third world countries. Currently, cervical cytology is widely regarded as the gold standard for cervical cancer screening in all developed countries. It is however not feasible to implement a systematic cytology based screening programme in a country like India. This is mainly due to severe restrictions on the availability of infrastructure, resources and funding. There is therefore a need to develop low cost screening strategies for cervical cancer. This will necessarily involve the use of a very simple technique that can be easily taught to and practiced by paramedical personnel in the rural areas. Such techniques will need to be cost effective while retaining adequate sensitivity and specificity to perform as practical screening techniques.

**Key words:** Cervical Cancer • HPV • Pap Test • Cytology

### INTRODUCTION

Cervical cancer is one of the most common types of cancer among women worldwide. The good news is that 92% of the cases can be detected (Table 1) and treated if a woman undergoes regular pelvic exams and Pap tests [1]. Many methods currently exist to detect cervical cancer. Cancer of the uterine cervix is a leading cause of mortality and morbidity among women worldwide. In India cervical cancer is still the most common cancer and occupies the top rank among cancers in women in most developing countries, constituting 34% of all women's cancers. Most cervical cancers arise at the squamo-columnar junction and transformation zone

between the columnar epithelium of the endocervix and squamous epithelium of the ectocervix where there is continuous metaplastic change. Since maximum metaplastic activity occurs at puberty and first pregnancy between 18-30 yrs of age, hence detected during active sexual life. All women are at risk of developing the disease, but several factors can increase the risk. The risk factors for cervical cancer are viral infections (HPV, HIV, HSV), multiparity, early initiation of sexual activity, multiple sex partners, smoking, low socioeconomic status, diet low in antioxidants, long term use of oral contraceptives and poor hygiene etc. Since early detection predicts better prognosis, one of the most effective ways of preventing and controlling cervical

Table 1: Key Statistics on India

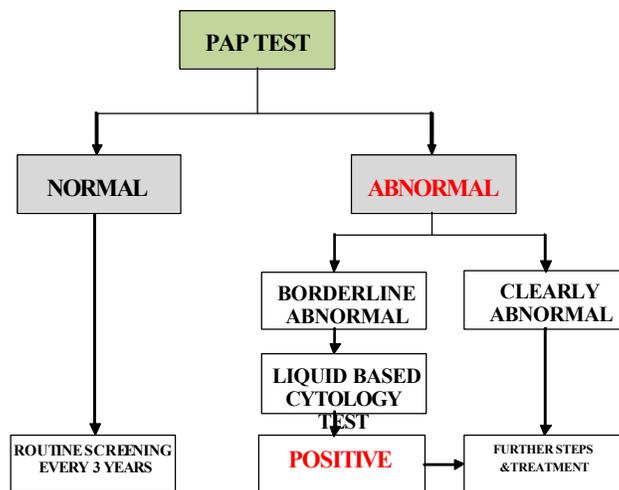
Population	
Women at risk for cervical cancer (Female population aged >=15 yrs)	366.58 millions
Burden of cervical cancer and other HPV-related cancers	
Annual number of cervical cancer cases	134420
Annual number of cervical cancer deaths	72825
Projected number of new cervical cancer cases in 2025*	203757
Projected number of cervical cancer deaths in 2025*	115171

• WHO Cervical cancer summary report update. September 15, 2010

S.No	Screening methods
1	Conventional exfoliative cervicovaginal cytology i.e. the cervical (Pap) smear
2	Pelvic Examination
3	Colposcopy
4	Liquid based cytology
5	Automated cervical screening techniques
6	Neuromedical systems
7	HPV testing
8	Polar probe
9	Laser induced fluorescence
10	Visual inspection of cervix after applying Lugol's iodine (VILI) or acetic acid (VIA)
11	Speculoscopy
12	Cervicography

cancer is regular screening and early diagnosis. Despite the fact that more than 80% of cervical cancer cases are in developing countries, only 5% of women there have ever been screened for cervical abnormalities (WHO 2006). Cervical screening is a way of checking women regularly for changes in the cells in the cervix. Invasive cervical cancer is largely preventable if precancerous lesions are detected by effective screening and then adequately treated. Several screening modalities are now available for early detection of cervical cancer and its precursor lesions. It is normally recommended that cervical cancer screening should begin three years after

vaginal intercourse is initiated and no later than the age of 21 [2]. For most women, the test is recommended every one to three years, depending upon the woman's age and history of abnormal results. For women who have a past history of an abnormal screening test or who have risk factors for cervical cancer, testing is recommended once per year (Picture 1). The Papanicolaou (Pap) smear is the most common screening method used to detect precancerous changes for squamous cervical cancer. However, other methods, i.e liquid based cytology and HPV testing are now the most advocated tests.



Picture 1: Frequency of PAP test

**Papanicolaou (Pap) Smear Test:** The most common form of diagnosis for detecting cervical cancer in its early stages is a procedure called a Papanicolaou test or Pap smear. This test is painless, normally takes less than 5 minutes to complete and can be performed in a doctor's office. Women who are 18 or older or who are sexually active are recommended to undergo annual Pap smear tests [3]. The procedure is performed while a woman is lying on her back on a table. The doctor will insert an instrument called a speculum inside her vagina before removing some cells from the cervix using a cotton swab or small brush. The cells are then sent to a laboratory where they are studied under a microscope to determine if any precancerous or cancerous cells exist. If the tests show any abnormalities, the patient will be asked to return to the doctor so an additional test can be performed. If the test results are negative, women can schedule an annual appointment (Picture 1) [4].

The value of the Pap test in cervical carcinoma screening is undisputed. Routine cytological screening by this method has resulted in a reduction in the cervical carcinoma mortality rate of close to 60% in women aged 30 and older. However, several limitations of conventional Pap smear were identified; sensitivity to detect cervical cancer precursors is less than 50%, inadequate transfer of cells to slide, in homogenous distribution of abnormal cells, presence of obscuring blood, inflammation or thick areas of overlapping epithelial cells. The occurrence of false-negative and unsatisfactory Pap smears has prompted the development of LBC and automated screening devices [5].

**Pelvic Examination:** A pelvic examination is also an important method of detecting cervical cancer. The exam is very similar to the Pap smear. A woman lies on her back while a doctor inserts a speculum into her vagina. A doctor will then examine a woman's vagina and surrounding organs both visually and manually. He will insert gloved fingers and gently feel the cervix and surrounding organs with his fingers, while his other hand presses gently on the patient's stomach [6].

**Colposcopy:** Colposcopy is magnified visual examination of the ectocervix, SCJ and endocervical canal using a special instrument called a colposcope. It may be accompanied by a biopsy of any abnormal-looking tissue is very similar to a Pap smear. A woman will lie on her back while a doctor inserts a speculum into her vagina. He will also apply a local anesthetic to her cervix as well as a special solution that will stain any abnormal cells

white. The doctor can then view the cells using the high-powered microscope to detect any abnormal cancerous cells [7]. It is used not as a screening test, but as a diagnostic test. Conization or Cone Biopsy, Endocervical Curettage, LLETZ/LEEP and imaging procedures are further techniques which may be applied when diagnosing cervical cancer.

**Liquid-Based Cytology (LBC):** All currently available cytology technologies rely on the visual analysis of exfoliated cells from the uterine cervix. Improvement of conventional cytological screening has been proposed by the introduction of molecular-based markers applied to liquid based cytology (LBC). It was developed to address the limitation of Pap smear and represents the first major change in preparation method for cervical screening samples. Instead of cells being smeared onto a glass slide, they are washed into a vial of liquid and filtered and a random sample is presented in a thin layer on a glass slide. DNA methylation changes occur very early in carcinogenesis and identification of appropriate DNA methylation markers in such samples should be able to distinguish high-grade squamous intraepithelial lesions (HSIL) from nonspecific cytology changes and the normal cervix [8]. The liquid based cytology (LBC) corresponds to a sampling where cells are put in suspension in a conversational liquid. For the clinician, the sample is made the same manner as that of the conventional smear by using a plastic brush, which can take the squamo-columnar junction and the endocervix, or by combining the use of a spatula and an endocervical brush. The taken material is then immediately rinsed in the bottle, which contains a fixative allowing transport to the laboratory. A part of the sizable brush can be left in the bottle. The clinician does not have to deal with any spreading, which is done at the laboratory. Currently, two technical methods, which use automats, were validated by Food and Drug Administration (FDA) and are used frequently [9].

One is proceeding by filtration and collecting cells vacuum-packed on a membrane with transferring cells on a glass (ThinPrep®, Cytoc®). The other is proceeding by centrifugation and sedimentation through a gradient of density (Surepath®, Tripath Imaging®). Cytoscreen System® (SEROA®), Turbitec® (Labonord®), CellSlide® (Menarini®) and Papsin® (Shandon®) techniques are centrifugation and sedimentation manual techniques, which do not use automate and do not require a FDA agreement [10].

Current methods that use LBC technology include:

**SurePath (formerly AutoCytePREP or CytoRich LBC):**

The SurePath method requires that the collection device be retained in the proprietary SurePath collection vial, which contains transport fluid, so that all cervical cells collected are sent to the laboratory. Vials are vortexed and centrifuged by laboratory personnel; all subsequent preparation of the sample and slide is automated using the Prepstain machine, which processes 48 samples at a time [11].

**Cytoscreen:** Cytoscreen is a manual method of sample preparation using a proprietary sample collection device (CYTOPREP) and transport fluid (CYTeasy). Samples are vortexed and a photometric reading taken to estimate the cellularity of the sample. An aliquot of the sample is centrifuged onto a glass slide that is then stained using normal laboratory procedures [12].

**Labonard Easy Prep:** Labonard Easy Prep is a manual method of sample preparation that uses a proprietary sample collection device (CYTOPREP brush) and fixative (CYTOscreen). An aliquot of sample fluid is placed in a separation chamber attached to a glass slide containing absorbent paper.

Cervical cells sediment onto the slide in a thin layer and slides are stained using normal laboratory procedures [13].

**Thin Prep:** ThinPrep provides a semi-automated (T2000) or fully automated (T3000) method of sample preparation. Cervical samples are rinsed with proprietary PreservCyt transport medium into a vial, which is then processed by the ThinPrep method using the T2000 or T3000 machine. The T2000 machine processes slides individually, while the T3000 machine is a fully automated device that can batch process up to 80 specimens per cycle. Subsequent staining and microscopic evaluation of the slides is conducted in a similar manner to a conventional smear test [14].

The quality of the evaluation of the performance of these technologies often was poor and rarely on the basis of histologically defined outcomes using randomised study designs. In general, the proportion of unsatisfactory samples is lower in LBC compared with conventional cytology and the interpretation of LBC requires less time. The cost of an individual LBC test is considerably higher, but ancillary molecular testing, such as high-risk HPV testing in the case of atypical squamous cells of undetermined significance (ASC-US), can be carried out on the same sample. The economic advantage

of LBC due to the reduction of recalls for a new sample. The advantage of LBC includes improved sensitivity and specificity since fixation is better and nuclear details are well preserved. There is, therefore, lower rate of unsatisfactory cervical samples. Another major advantage of LBC is that the residual specimen can be used for ancillary testing like Immunocytochemistry and detection of HPV DNA [15].

**Automated Screening Technology:** The effectiveness of any cervical cancer screening program that relies on cervical cytology is the quality control of the cytological review of Pap smears. This is essential for reducing the false positives and false negatives that invariably result from inter- and intra- observer variation [16]. Automated screening techniques have recently been developed that can not only perform this quality control rescreening but also can be used for primary screening of cervical smears. The Autopap300 (TriPath Imaging, Burlington NC) and PAPNET (Neuromedical systems) are the two automated screening techniques that rely largely on neural network technology and are based on the computerized imaging and identification of abnormal cervical cells. Among this only the Autopap300 is approved by the USFDA for primary and secondary cervical screening while the PAPNET is only approved for secondary screening [17].

**Hpv-Dna Testing:** The etiopathological role of HPV in the development of cervical cancer has been proved beyond doubt. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59 and 68 are known to be frequently associated with HSIL and invasive cancers of the cervix [18]. Testing for the presence of HPV-DNA in the cervical cells is thus a potentially useful screening method, which could be incorporated in cervical cancer screening programs. There are various techniques available for HPV-DNA testing of which Southern Blot hybridization is regarded as a laboratory gold standard. This is however unsuitable for clinical use as it is laborious, tedious and requires fresh tissue. The specimen for HPV-DNA testing can be obtained in two ways, either by using a cell suspension from liquid based cytology or by using the endocervical cytobrush [19].

**Visual Inspection of the Cervix with Acetic Acid (VIA):**

The technique is very simple and consists of an examination of the cervix after acetic acid application. After obtaining the clinical history and performing a general examination, the cervix is exposed using a bivalve speculum. A 4% dilute solution of acetic acid is then

applied to the cervix and any excess liquid is aspirated from the posterior vaginal fornix. The cervix is inspected after two minutes. Lesions which stain acetowhite are regarded as positive for VIA. Those with dull white plaques and faint borders are considered low grade VIA while those with sharp borders are considered high grade VIA. The test is regarded as being negative if no acetowhite lesions are detected [20]. Studies have shown that VIA is a reliable, sensitive and cost effective alternative to conventional Pap smear testing, particularly in low resource settings. VIA is better than Pap smear for identifying CIN—especially if a woman is tested only once in her life. VIA is simple to perform and provides an immediate result without expensive equipment.

**Visual Inspection with Lugol's Iodine (VILI):** VILI is also promising as a visual method to screen for cervical cancer. After the woman's cervix is examined using VIA, the cervix is painted with Lugol's iodine solution and examined again with the naked eye. The small high-grade lesion is easier to see within the larger low-grade area. Normal squamous epithelial cells have substantial stores of glycogen.

Glycogen stains mahogany-brown with iodine solution. Abnormal areas of squamous epithelium (CIN or inflammation) do not contain glycogen to the same extent and do not stain brown [21]. VILI is more accurate and more reproducible than VIA and better than a Pap smear for identifying CIN. VILI is simple to perform and provides an immediate result without expensive equipment.

**Speculoscopy:** Speculoscopy involves inspection of the cervix following the application of 5% acetic acid with chemiluminiscent light and a low power magnification.

**Cervicography:** Cervicography involves taking photographs of the cervix using a special camera following the application of 5% acetic acid during a routine pelvic examination and Pap smear collection. The photographs are then developed and the slide is projected on a 2x2 meter screen and read by an expert in colposcopy.

**Polar Probe:** This technology is based upon the fact that the tissue impedance to electrical stimulation differs between normal and abnormal tissues. Investigators have tried to utilize spectral and electrical stimulation of the cervical tissues as an adjunct to conventional Pap smear testing.

**Laser Induced Fluorescence:** Various investigators have shown that low powered laser illumination can induce endogenous tissue fluorescence. This depends upon the chemical and morphological composition of individual tissues. The spectroscopic difference if detectable can be used to differentiate normal and diseased tissues [22].

**Computer Imaging:** The diagnosis of precancerous changes is primarily a task of visual discrimination and sorting of graphical information. Recently there has been a lot of focus on the use of computers to assist this process. This is very similar to cervicographic technics except that a computer replaces the colposcopy expert. However a lot of research needs to be done to critically evaluate this technology before it can be incorporated into a screening program.

**Comparison of Visual Inspection with Acetic Acid and Cervical Cytology to Detect High-Grade Cervical Neoplasia:** As a point-of-care clinical test, VIA is easy and inexpensive to perform, can be taught to non physician health workers and can link screening and diagnostic or treatment interventions in the same clinic visit. On the other hand, there are significant real-world challenges in implementing cytology- based screening in resource-limited settings. Adequacy of samples is hampered by challenges in sample collection and slide preparation. Even in the hands of highly qualified pathologists, cytological interpretation is precariously modest in its detection rate and sensitivity. Besides, the costs and inconvenience of multiple clinic visits and the resulting poor compliance in cytology-based screening programs are significant barriers to successful cervical cancer control for most resource-limited settings.

**Conventional Pap Smear and Liquid Based Cytology for Cervical Cancer Screening:** Cervical cytology was introduced by George Papanicolaou into clinical practice in 1940. In 1945, the Papanicolaou smear received the endorsement of the American cancer society as an effective method for the prevention of cervical cancer. Center of cytology in Vancouver, British Columbia published data which confirmed that cytologic screening leads to a reduction in the rate of invasive cancer of the uterine cervix. Park *et al.* established that the sensitivity of the conventional Pap smears for the detection of cervical cancer precursors was less than 50%. Several limitations of conventional smear were identified including inadequate transfer of cells to slide, in homogenous distribution of abnormal cells, presence of obscuring

blood, inflammation or thick areas of overlapping epithelial cells. Liquid based, thin layer technology was developed to address the limitation of Pap smear. More than 5,00,000 subjects have been studied with a preponderance of data indicating a significant benefit of liquid-based, thin layer technology in the detection of cervical cancer precursor lesions and in the improvement of specimen adequacy.

The Pap smear has been utilized for cervical cancer screening for more than 50 years. Despite being credited with a 70% reduction in mortality for cervical cancer, the false negative rate is still a cause for concern. It is widely acknowledged that two third of the overall false negative rate can be attributed to sampling errors. Liquid based cytology has been developed to address the sampling problems of conventional Pap smear. The present work was done to evaluate the liquid based cytology and to compare the sensitivity of the same with conventional Pap smear. Liquid based cytology is strongly advocated in the best interest of public health, by improving the quality of the sample and reducing the likelihood of false negative cytology results. Thus it will significantly improve early detection and treatment of cervical lesions.

**Liquid-Based Cytology vs Conventional Cytology in Detecting Cervical Cancer:** The advantages of using liquid-based cytology are not adequately measured by the direct test cost alone. Although it remains more expensive on a per-test basis than conventional cytology, there are several benefits of liquid-based cytology for the laboratory and the patient, some of which can save downstream costs. Liquid-based cytology slides are easier and quicker to interpret; yield fewer inadequate tests; and, in the case of an equivocal cytology result, permit human papillomavirus (HPV) testing without the need for another patient sample. Cost-benefit analyses that consider not only direct test cost but also the ramification costs of using one technology over another are needed to provide true cost comparisons; to our knowledge, no such analysis in the US setting has been performed. It is true that nearly universal acceptance of liquid-based cytology occurred before the completion of definitive comparative effectiveness trials. Ideally, for screening tests designed for widespread general use, public health practice should be based on the strongest possible evidence. In reality, this sequence is sometimes not followed. As we stated in our Editorial, the choice of conventional cytology or liquid-based cytology is now less important than comprehensive re-examination of cervical cancer screening programs, with increased consideration of HPV-based technologies. Prophylactic

vaccines will reduce the efficiency of screening by any method, while introduction of HPV testing is likely to lead to increased screening sensitivity that should permit lengthened screening intervals. It is not yet clear how to optimally combine prevention and screening options. The further evolution of screening practices should follow high-quality data on comparative effectiveness.

## CONCLUSIONS

Over the past few years, in most industrialised countries women with cervical cancer have benefited from improved imaging techniques, better treatments (including chemoradiotherapy) and more conservative surgical approaches. In low resource settings-where facilities for radiology, chemoradiotherapy and supportive care are limited or unavailable-it is important to identify which resources fill healthcare needs most effectively and to consider alternative approaches.

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