# EVALUATION OF SECOND-GENERATION LIQUID-BASED CYTOLOGY SYSTEM FOR THE DETECTION OF CERVICAL ABNORMALITY

**A Thesis submitted to the University of Manchester for the degree of Doctor of Medicine in the Faculty of Medical and Human Sciences**

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**By**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **NHSCSP** | National Health Service cervical screening programme |
| **NICE** | National Institute for Health and Clinical Excellence |
| **LBC** | Liquid-based cytology |
| **SP™** | SurePath™ |
| **TP™** | ThinPrep™ |
| **CS 120™** | CellSolution 120™ |
| **PASA** | NHS Purchasing and Supply Agency |
| **CEP** | Centre for Evidence based Purchasing |
| **MCC** | Manchester Cytology Centre |
| **GMEC** | Guildford Medical Evaluation Centre |
| **rpm** | Revolutions per minute |
| **DGC** | Density gradient centrifugation |
| **BSCC** | British Society for Clinical Cytology |

## ABSTRACT

Liquid-based cytology (LBC) has replaced conventional smears in the UK. The National Institute for Health and Clinical Excellence (NICE) recommended the use of LBC in 2003. ThinPrep™ (TP) and SurePath™ (SP) LBC systems were adopted for use in the National Health Service Cervical Screening Programme (NHSCSP) in the UK. NICE recommended further review of any other technologies or other liquid-based cytology systems in the future. For any second-generation LBC systems to be considered for cervical screening in the NHSCSP, there must be an evaluation of technical requirements and clinical data relating to their sensitivity, specificity and the percentage of inadequate samples.

The objective of the work undertaken for this thesis was to provide evidence to enable an informed decision on the use of second-generation liquid-based cytology systems for cervical screening in the UK. The decision to accept the second-generation LBC system in the NHSCSP is based on its reliability, clinical effectiveness and cost implications. This work will determine the reliability, microscopic quality and reproducibility of slides of the second-generation LBC system, and the results of this work will form the platform for progression to the clinical evaluation of the system.

Initially, four second-generation LBC systems were considered suitable for evaluation. They were Seroa CYTO-screen, Shandon Papspin, LGM Liqui-PREP and CellSolution 120. However, the specifications of only one system (CellSolution 120™) met NHSCSP technical requirements to start the evaluation. One hundred random, electronically generated colposcopy patient samples were used to assess the technical reliability of the CellSolution 120™ system. The technical evaluation consisted of pre-phase I and phase I. The results of these phases will decide whether the CS 120™ liquid-based cytology system could be carried further for clinical evaluation (phase II) or not.

This study was sponsored by the NHS Purchasing and Supply Agency (PASA), the Centre for Evidence based Purchasing (CEP) on behalf of the NHSCSP. The Manchester Cytology Centre (MCC) was selected as the site for evaluation of CellSolution 120™ and the project was managed by Guildford Medical Device Evaluation Centre (GMEC) on behalf of CEP.

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## FOREWORD

The findings in this thesis are the results of original work. The assistance of others is acknowledged below. No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other University or institute of learning. This work was presented at the 17th International Congress of Cytology, Edinburgh, in May 2010 by the Author. The abstract of the presentation was published in the Acta Cytologica 2010 May-Jun; 54 (3 Suppl): 369-528.

## DEDICATION

I would like to dedicate this thesis to my beloved and late mother, Mrs. Jyotsna K. Merchant. She passed away suddenly on the 16th March 2010. I express my gratitude to her for enormous encouragement, love and support throughout my life.

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## THE AUTHOR

The author was born in Ahmedabad, Gujarat, INDIA in 1977. Following the secondary education at Udgam School for Children, she graduated M.B.B.S. from M. P. Shah Medical College, Saurastra University in 2001. She completed basic pathology training and graduated MD (Pathology) from Rajkot Medical College, Saurastra University in 2004. The author came to England in 2005, where she did an observership in Histopathology and Cytopathology. At present, she has joined as a lecturer in Pathology at the University College Dublin.

# INTRODUCTION AND REVIEW OF LITERATURE

### 1.1 Establishing the importance of the topic

Cervical cancer is the most common cancer seen in women in developing countries (1). It is the twelfth most common cancer among women in the UK (2). The difference in the cervical cancer rate between the UK and developing countries is due to the introduction of computerised call and recall system in the “National Health Service Cervical Screening Programme” in the UK in 1988. Initially this screening programme, which screened all the eligible women in the country, was known as the National Health Service Cervical Screening Programme National Coordinating Network (NHSCSP NCN). Then, in 1994, it came to be known as the National Health Service cervical screening programme (NHSCSP) and later on from 1997 this cervical screening programme was carried out only in England, while Scotland, Wales and Northern Ireland initiated their own cervical screening programmes (3). The aim of NHSCSP is to reduce the number of women who develop and die from invasive cervical cancer by regularly screening women at risk so that precancerous changes (moderate (cervical intraepithelial neoplasia (CIN) 2) (22%) and severe dyskaryosis (CIN 3) (14%)) (4), which may otherwise develop into invasive cancer, can be identified and treated accordingly (5).

The cervical screening programme is an expensive programme, which requires proper infrastructure with trained clerical staff, sample collectors, laboratory staff, transport system and gynaecologists to treat patients with abnormal cytology. Developing countries cannot afford such an expensive screening programme. Therefore, there is a high mortality rate of cervical cancer in young women worldwide (6). However, in Europe, where screening is much more common, the scenario is different. Six European countries started

screening in the 1960s and ten countries or regions started at least a pilot programme by 2003 (7).

## 1.2 Importance of cervical screening programme

The population screening programme in British Columbia during the 1960s was the first to demonstrate to the world that both the incidence and mortality rate from cervical cancer can be reduced by implementation of a screening programme (8). Cervical screening has been available in the UK since 1967 (2). However, it was not in wide-spread use in the UK until the 1980s. Therefore, the mortality rate from cervical cancer remained nearly the same till 1988 when a systematic national programme for screening women aged 20-64 years was introduced in this country. The programme invited women for screening regularly at least every 5 years (6).

Quinn et al (9) concluded that improvement in the screening programme has led to a 35% fall in the incidence of invasive disease and there is a reduction in mortality in women under 54. The programme is believed to have prevented 800 deaths in 1997, but not in women over 54 (9). The incidence of cervical cancer has fallen sharply from 16 per 100000 in 1986 to 9.3 per 100000 in 1997 and the mortality rate is currently falling by 7% per year (10). It is estimated that 4500 lives are saved per year by the cervical screening programme (11). Only 413 deaths of women aged 25-64 due to cervical cancer were reported in England in the year 2007 (12). The cervical cancer death rate continues to decline by approximately 4% every year in the U.S.A (13).

### 1.3 Screening age variability across the world

Across the world, the age group screened differs. For example, in Australia the age range is 18-69 years. Routine screening is carried out every two years. Women over 70 years who have never had a smear test, or who request a smear test, are also screened (14). In New Zealand regular cervical smear tests every three years are recommended for women from the age of 20 till 70 if they have ever been sexually active (15). In Hong Kong, women are screened from 25 years to 64 years of age (16). The American Cancer Society recommends a woman to go for a regular cervical smear three years after starting sexual intercourse. It recommends a conventional smear test every year or a liquid-based cytology smear every two years. Women aged 30 years or more with three normal smears in a row can be screened every 2-3 years with a human papilloma virus DNA test. Women 70 years of age or older who have had 3 or more normal cervical smear tests in a row and no abnormal smear test results in the last 10 years may choose to stop having cervical cancer testing in America (17).

The screening age also varies in the UK. Since 1993 in England, women aged 20 to 64 year have been screened regularly at three to five year intervals. In 2007, the policy was refined with three-yearly screening for 25-49 year-old women and five-yearly screening for 50-64 year-old women. In Northern Ireland and Wales, women aged 20 to 64 years are screened regularly, while in Scotland women are screened from 20 to 60 years of age. However, studies have shown that cervical cancer is rare under the age of 25 years and also changes in the cervix are common in younger women, which makes screening less cost effective in women under 25 (18). Women aged 65

or more are only screened if they were either not screened after the age of 50 or have recent abnormal tests (5).

2007-2008 statistics from the NHSCSP also show the percentage of test results of severe dyskaryosis or worse is highest in women aged 25-29 (1.4%), while it is lowest in women aged 60-64 years (0.2%) (12) (results are taken from GPs and community clinics) (shown in Figure 1).

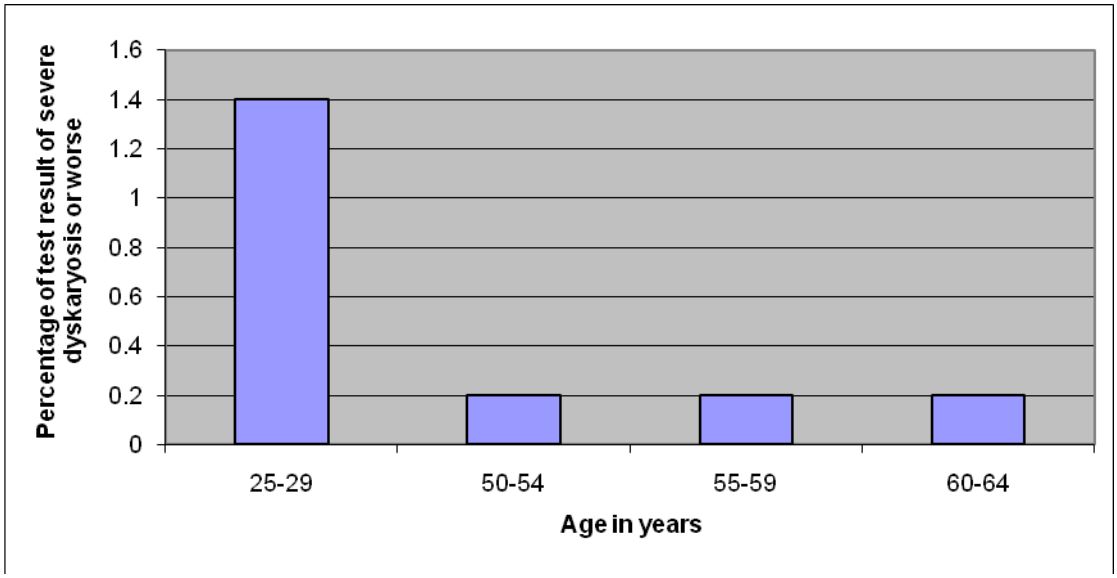



Figure 1: Women by age with test result severe dyskaryosis or worse as a percentage of all women with adequate test results

### 1.4 Method of obtaining a conventional cervical smear

There are different methods of obtaining and processing cervical smear samples. In the conventional method, a woman lies down on a couch with the legs apart and then either a doctor or a nurse inserts a speculum to view the cervical os. A cervical sample is obtained using a wooden spatula or plastic brush. The spatula or brush is rotated twice in the clockwise direction to scrape the transformation zone of the cervix. The transformation zone is the area of cervix where the columnar epithelium is transformed to squamous epithelium due to vaginal acidity and the area where most abnormal changes occur. Therefore the transformation zone needs to be sampled in every case (19). The spatula or brush containing a sample is smeared on a glass slide and rapidly fixed with an alcohol spray to prevent the cells from degenerating. Poor fixation will not allow the cells to stain properly, making the smear difficult to assess.

Later, the glass slides are sent along with respective request forms to the laboratories for Papanicolaou (Pap) staining and assessment under a microscope by trained staff. Dr. George Papanicolaou developed the staining procedure to see the cervical cells in 1930. Figure 2 below shows a stained conventional smear and a wooden spatula which is used to obtain the sample.


Figure 2: A wooden spatula and conventional Pap smear prepared by spreading the cells on a slide

### 1.5 Reasons for newer method in cervical screening

The conventional smear method results in many false negative diagnoses either due to sampling or screening errors. Sampling errors occur when all the abnormal cells may not be scraped off, abnormal cells may stick on the wooden spatula rather than being smeared on the slide or the cells transferred to the slide may not be representative of an abnormality. Screening errors occur if the smears are too thick, if the squamous cells are obscured by polymorphs, red blood cells and debris, if the screeners are tired and if there is difficulty in interpretation of cells. Thick smears are difficult to interpret; polymorphs and mucus obscure the abnormal cells and also due to drying artefacts. Many conventional smears were reported inadequate due to sampling or screening deficiences. Inadequate results raised anxiety amongst women and also compounded the cost per cervical sample in the National Health Services. Allen et al raised concerns about false negative smears in 1996 and suggested that automated screening and human papilloma virus testing would lead to an improvement in the cervical screening programme (20). This has eventually led to a call for a change in practice in cervical screening but it has taken more than 10 years to implement. The US Food and Drug Administration approved two Liquid-based Pap tests, namely the ThinPrep$^{TM}$ in 1996 and the SurePath$^{TM}$, formerly known as AutoCyte PREP or CytoRICH in 1999 as a replacement for the conventional smear test. The liquid-based Pap test involves the use of Cervex-Brush® (Rover's Medical Devices BV, Oss, The Netherlands) (shown in figure 3) or a combination of a plastic spatula and endocervical brush to obtain a cervical sample (sample from transformation zone). The head of the spatula or brush is either kept in

the vial containing preservative by detaching the head of the brush or removed after rinsing the spatula or brush thoroughly in the preservative vial.

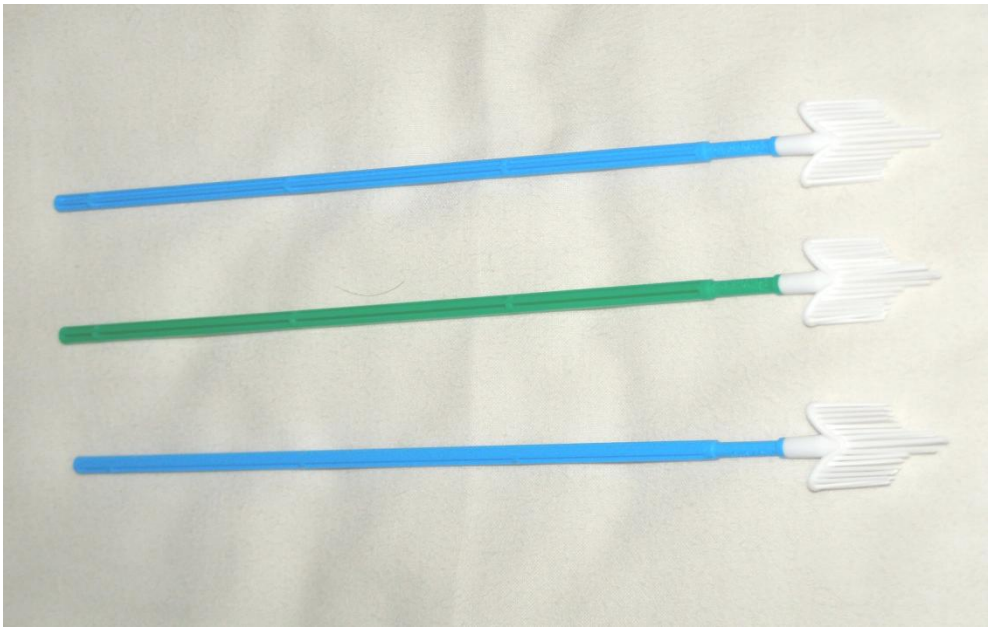


Figure 3: Cervex-Brush® used in liquid-based cervical cytology

The cervical sample is automatically processed from a liquid medium in a laboratory, where excess mucus and polymorphs are removed and a representative thin layer of epithelial cells is deposited on a glass slide in a circle. The major differences between conventional and liquid-based slides are shown in table 1 below.

Table 1: Major differences between conventional smear and liquid-based slides

| Conventional smears | Liquid-based slides |
|---|---|
| Heterogeneous presentation | Homogeneous presentation |
| Graphic cell presentation | Random cell presentation |
| Variable fixation | Uniform fixation |
| Thick uneven groups- need frequent focusing | Uniform thin layer |
| Dirty background | Clean background |
| 300-500 k cells/slide | 50-70 k cells/slide |
| Less nuclear detail visible | More nuclear detail visible |
| No residual sample | Residual sample can be used for HPV and molecular testing |

### 1.6 Efficacy of liquid-based cytology systems

There have been various studies undertaken around the world to establish the efficacy of liquid-based cytology systems. The majority of conventional smears have now been replaced by liquid-based cytology in the US (21). The US Food and Drug Administration (FDA) approved the ThinPrep™ Pap Test as "significantly more effective" at detecting precancerous cervical cells (22). The US FDA also approved the SurePath™ Pap Test concluding that 64.4% high-grade squamous intraepithelial lesions or worse are detected by this testing system and it significantly reduces unsatisfactory samples (23). A study in Australia suggested that the ThinPrep™ system was also cost effective when compared to conventional smears (24). Bergeron recommended both the conventional smears and liquid-based cervical cytology in Europe as he did not find any evidence suggesting higher accuracy of liquid-based cytology (25). In the UK, the National Institute for Health and Clinical Excellence (NICE) chose three laboratories, one each in Bristol, Norfolk and Norwich and Newcastle upon Tyne, to start a pilot study on liquid-based cervical cytology (LBC) in 2001. The purposes of this study were to determine the sensitivity of LBC, its cost effectiveness and the practical implications of introducing it into the NHS cervical screening programme (26). The laboratories at Bristol and Norfolk and Norwich used ThinPrep™, while Newcastle upon Tyne used the SurePath™ liquid-based cytology system. A total of 100,000 routine screening cervical samples were collected, processed and reported.

## 1.7 National Institute for Health and Clinical Excellence (NICE) recommendations

The results of the LBC pilot study were published in 2003 and showed a definite reduction of inadequate smears (from 9% to 1-2%), increased laboratory efficiency, reductions in the back-log of samples and overall cost. Additionally, the results showed that the sensitivity of detection of high-grade dyskaryosis was similar to conventional smears (27). Moreover human papilloma virus DNA testing and additional automation can be integrated into the programme with the help of liquid-based cervical cytology systems. On 22$^{nd}$ October 2003, NICE recommended liquid-based cervical cytology to be used in National Health Service cervical screening programmes in England and Wales as the primary means of processing and screening cervical samples. NICE suggested the conversion to liquid-based cytology from conventional smear tests in NHS cervical screening programme within five years. Scotland accepted liquid-based cervical cytology as a replacement for conventional smear tests in April 2002. It was the first European country to introduce liquid-based cervical cytology (28). Slides prepared from the liquid-based cytology methods are shown in figure 4 below.


Figure 4: Conventional smear (the smear throughout the slide), the ThinPrep™ and SurePath™ (well-circumscribed) samples

NICE recommended three liquid-based cytology machines, i.e. SurePath™ where 48 samples are processed at a time, in 2.10 hours, the ThinPrep™ T2000 (semi-automated) where 1 sample can be processed in 4 minutes and ThinPrep™ T3000 (fully automated) where 80 samples are processed in one cycle (2.5 hours). In the SurePath™ system, vials are vortexed and centrifuged by laboratory staff and then the sample is prepared and stained by Prep stain (a component of the SurePath™ machine). In the ThinPrep™ (T2000 and T3000) system, the sample is prepared by the machine and then stained either manually or by a separate staining machine using the stain of an individual laboratory (29).

The National Institute for Health and Clinical Excellence also endorsed the recommendations made by companies (ThinPrep™ and SurePath™) regarding a particular technique in obtaining a cervical sample for the liquid-

based method. The central bristles of the brush should be inserted into the endocervical canal and the outer bristles should remain in contact with the ectocervix (as shown in figure 5) (30).

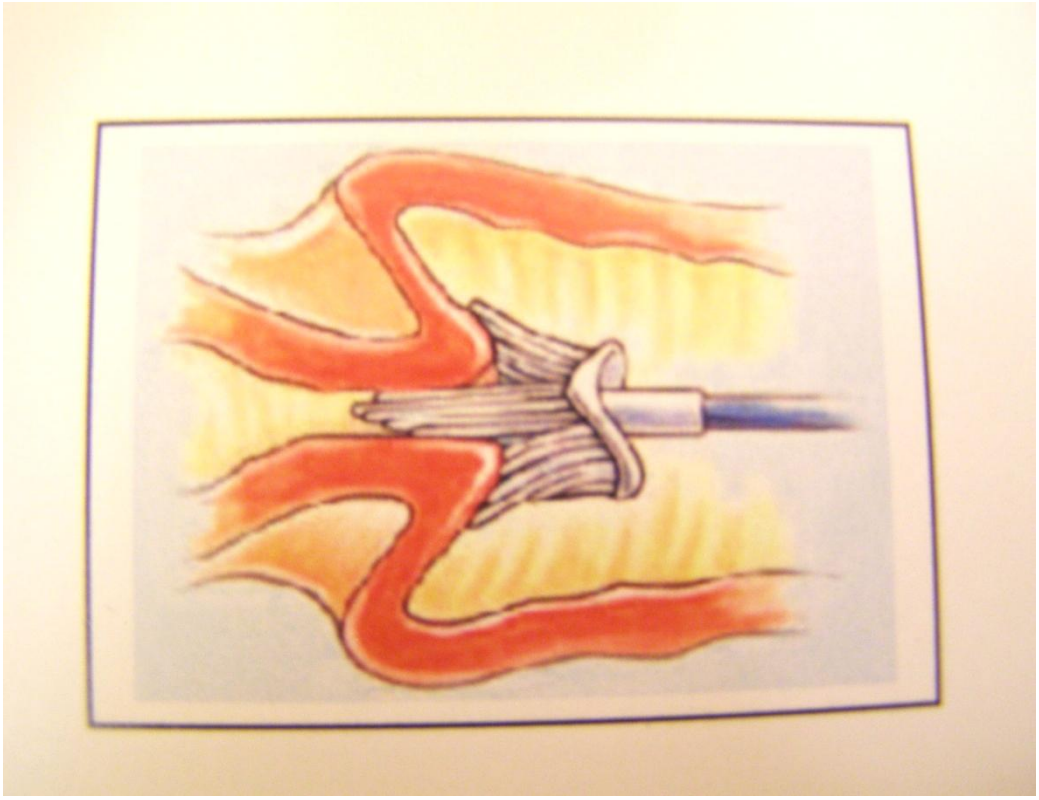


Figure 5: The inner bristles of a brush are inserted into the endocervical canal, while outer bristles remain in contact with the ectocervix. (Figure adapted from Central Manchester and Manchester Children's University Hospitals, NHS Trust)

A sample is obtained by rotating the brush inside the canal 5 times in a clockwise direction. This sample is either rinsed directly to remove the cells from the brush (by pushing the brush into the bottom of a vial at least 10 times and keeping the bristles apart) (ThinPrep™) or the head of the brush (SurePath™) is broken off and placed into the preservative vial (as shown in figure 6).

Figure 6: The head of the cervex brush is detached and kept into the preservative vial for the SurePath™ liquid-based cytology system. (Figure adapted from Central Manchester and Manchester Children's University Hospitals, NHS Trust)

NICE also recommended a review of newer or second-generation liquid-based cytology systems other than ThinPrep™ and SurePath™ (30).

## 1.8 Overview of ThinPrep™ and SurePath™ liquid-based cytology systems

The principles of the two liquid-based cytology systems (ThinPrep™ and SurePath™) are different. The ThinPrep™ system uses an ultra filtration method, while the SurePath™ system is based on the density gradient principle to obtain thin-layer circular 19mm and 13 mm smears respectively on the slide. The ThinPrep™ preservative vial, Cervex-Brush® and a sample are shown in figure 7 and the SurePath™ vial, brush and sample are shown in figure 8 below.


Figure 7: The ThinPrep™ vial containing methanol as a preservative, Cervex-Brush® and 19 mm circular sample on the slide

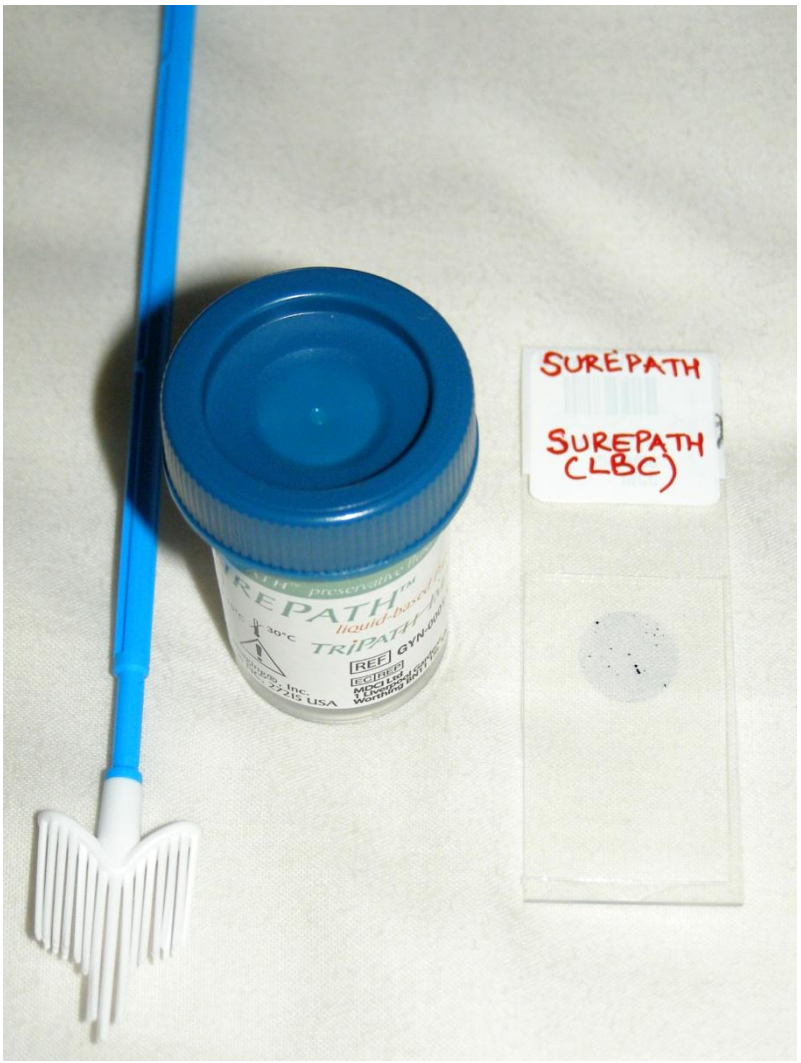



Figure 8: The SurePath™ vial containing ethanol as a preservative, Cervex-Brush® and 13 mm circular sample on the slide

The major differences between the two liquid-based cytology systems are listed in table 2 below.

Table 2: Major differences between ThinPrep™ and SurePath™ liquid-based cytology systems

| **ThinPrep™** | **SurePath™** |
|---|---|
| Methanol as a preservative | Ethanol as a preservative |
| Cervex-Brush® head is rinsed in the vial | Cervex-Brush® head is left in the vial |
| 1.9 cm diameter circle of cells | 1.3 cm diameter circle of cells |
| Positively charged slides are used | Pre-coated slides are used |
| T2000- printed barcode label is pasted manually<br>T3000- printed barcode is automatically pasted on slide | Printed barcode labels are pasted manually |
| Individual laboratory's stain can be used, slides not stained by the machine | Company's stain has to be used, slides are stained by the machine |
| Any cover slip- 24x40 mm to be used | Company provided cover slip 22x50mm to be used |
| Waste to be collected in Genta containers | Waste is incinerated |
| Residual samples can be stored for a shorter period (4 weeks) | Residual samples can be stored for a longer period (6 months) |
| Only vials to be stored | Vials and test tubes to be stored |
| Less space required for sample storage and the machine | More space required for sample storage and the machine |
| Cell filtration method is used | Cell enrichment process is used |

Table 2: Major differences between ThinPrep™ and SurePath™ liquid-based cytology systems (continued)

| **ThinPrep™** | **SurePath™** |
|---|---|
| T2000- labour intense<br>T3000- labour free | Labour intense process |
| Well-demarcated edge- no drift | Drifting of cells seen |
| Holes between cells | No holes between cells |
| Less 3 dimensional effect seen | More 3 dimensional effect seen |
| Less need to use high-power | Need to use high-power more often |
| Metaplastic cells – a difficult area | Hyper chromatic crowded cell groups- a difficult area |
| Maximum capacity per year<br>T2000- 30,000 samples<br>T3000- 60,000 samples | Maximum capacity per year<br>72,000 samples |

## 1.9 Troubleshooting in ThinPrep™ and SurePath™ liquid-based cytology systems

No system is perfect in its application. Table 3 below shows the major problems in ThinPrep™ and SurePath™ liquid-based cytology systems.

Table 3: Troubleshooting in ThinPrep™ and SurePath™ liquid-based cytology systems

| **ThinPrep™** | **SurePath™** |
|---|---|
| Only positively charged slides are required | Pre-coating of slides if improper, results into either patchy or no cells |
| T2000- barcode labels to be manually pasted on the slides<br>T3000- barcode labels pasted by the machine | Barcode labels to be manually pasted on the slides |
| Lubricant artefacts are found- aqua gel, KY gel and powder glove granules are seen | Lubricant artefacts are found- aqua gel, KY gel and powder glove granules are seen |
| | Z max hole-because of too low vacuum tube |
| | Unsecured settling chamber results into two rings of cells |
| | Drying artefact (Chico effect) occurs when the settling chambers are removed together rather than individually while applying coverslip |

Table 3: Troubleshooting in ThinPrep™ and SurePath™ liquid-based cytology systems (continued)

| **ThinPrep™** | **SurePath™** |
|---|---|
| Cells are drifted when much pressure is applied while coverslipping | Cells are drifted when much pressure is applied while coverslipping |
| Orange colour of cytoplasm is not lost | Orange colour of cytoplasm is lost |
| Cell crowding is less frequent | Cell crowding with three dimensionality is seen |
| Metaplastic cells versus high-grade and bland cell dyskaryosis are difficult areas to interpret | Small atypical cells and hyper chromatic crowded cell groups are difficult to interpret |
| Too cellular samples produce just a ring of cells and results in an inadequate report. However, the sample can be reprocessed | |
| Compression artefact occurs at the edges of the ring which produces drying artefacts- swollen cells and large nuclei resulting in false positive results | |
| Gaps (holes) between the cells are found | |
| Lysed blood cells can obscure the squamous cells | |

Table 3: Troubleshooting in ThinPrep™ and SurePath™ liquid-based cytology systems (continued)

| **ThinPrep™** | **SurePath™** |
|---|---|
| Hybrid capture test 2 for HPV testing cannot be performed if acetic acid is used to lyse the red blood cells | |

### 1.10 Cost implications per liquid-based cytology sample

The cost per SurePath™ LBC and ThinPrep™ liquid-based cytology sample in a laboratory processing 60,000 samples per year is approximately £20.76 and £23.15 (for T2000) and £22.99 (for T3000) respectively (27). The summary of total costs per conventional cytology sample and LBC sample are shown in the table below.

Table 4: Summary of total cost per conventional cytology sample and LBC sample:

| **Items** | **Conven-tional smear** | **T3000** | **T2000** | **SurePath™** |
|---|---|---|---|---|
| Smear taker staff cost | £7.66 | £4.93 | £4.93 | £4.93 |
| Administration cost | £3.00 | £3.00 | £3.00 | £3.00 |
| Preparation equipment cost | £0.04 | £0.52 | £0.36 | £0.22 |
| Preparation staff cost | £0.02 | £0.06 | £0.41 | £0.20 |
| Consumable cost | £0.27 | £4.07 | £4.07 | £2.00 |
| Smear reading cost | £2.26 | £1.99 | £1.99 | £1.99 |
| Other laboratory cost | £8.42 | £8.42 | £8.42 | £8.42 |
| TOTAL | £21.68 | £22.99 | £23.15 | £20.76 |

### 1.11 Highlighting a problem or controversy in the field of study

The major problem with the current liquid-based cytology systems (i.e. SurePath$^{TM}$and ThinPrep$^{TM}$) is their cost implications.

The National Health Service Cervical Screening Programme decided to explore other liquid-based cytology systems, which are at least equally or more efficient than the currently used LBC systems in terms of clinical and cost effectiveness.

The new second-generation liquid-based cytology systems are:

- CellSolution 120 (Synermed)
- LiquiPrep (LGM)
- PapSpin (Shandon)
- Cytoscreen (Seroa)
- Turbitec (Labonord)
- CellSlide (Menarini)
- MonoPrep Pap Test (MPPT)
- MonPrep2 (MP)

## 1.12 Various studies in this field highlighting a knowledge gap in the field of study

The literature search has shown published studies on this subject.

Joonseok Park et al (31) reported that Liqui-PREP™ is more sensitive than conventional smears and more cost effective than ThinPrep™ and SurePath™. Park et al verified Liqui-PREP™ cytology results against histology results. However, the authors did not mention the number of samples processed in one cycle and cost per Liqui-PREP™ sample. The authors also did not state whether an automated machine was used. Neither did they compare with the current liquid-based cytology systems (ThinPrep™ and SurePath™). In their study none of the samples were processed by both techniques, namely the conventional and the Liqui-PREP™

Jae Soo Koh et al (32) evaluated CellPrep® (CP) liquid-based cytology to find a cost effective and automated alternative for cytology specimens. The authors in this evaluation did not avoid collection bias for the samples. They have compared the results of a newer liquid-based cytology system with the ThinPrep™ results and not with the histology results. Cell adherence and stain ability were problems encountered in the study.

Cytoscreen System® (SEROA®), Turbitec® (Labonord®), CellSlide® (Menarini®) and Papspin® (Shandon®) are manual techniques (25). These liquid-based cytology techniques do not require US Food and Drug Administration (FDA) approval as they are not automated (25). Bergeron C et al (33) reported that the Cytoscreen System® (SEROA®) produced high quality slides and detected more squamous lesions than conventional Pap smears. However, only detection of atypical squamous lesions of undetermined significance (Borderline category in the UK, BSCC terminology)

was statistically significantly improved over conventional smears. Weynard B et al (34) stated that Papspin® (Shandon®) samples with the result of satisfactory, but limited by category (no equivalent terminology in the UK) had improvement in their presentation. Moreover, human papilloma virus test could be done easily on the Papspin® (Shandon®) samples. These evaluated techniques are compared to conventional smears rather than existing liquid-based cytology systems and none of the systems are evaluated for diagnostic accuracy by comparing them with the 'gold standard' (histology). Christian Garbar et al (35) evaluated the efficiency of inexpensive liquid-based cytology systems: Papspin® (Shandon®) and Turbitec® (Labonord®). The author reported that these two liquid-based cytology systems slides were similar to SurePath™ in reading and that cell debris, inflammatory cells, lactobacillus and blood were present, but they did not obscure the cells. There was no statistical significance between the results of both the systems. However, the sample size in this research was low (51 samples for Papspin® (Shandon®) and 215 samples for Turbitec® (Labonord®).

NAM Jong Hee et al (36) evaluated the accuracy of a newer liquid-based cytology system, the modified MonoPrep2 (MP) by comparing it with the ThinPrep™ technique. The author concluded that MP was less sensitive and more specific than the ThinPrep™ system. The authors also stated that the modified MonoPrep2 is a cost effective alternative to the currently expensive liquid-based cytology system. However, this is a manual technique.

Edmund S et al (21) determined the efficacy for a newer liquid-based cytology system, MonoPrep Pap Test (MPPT). The authors compared the newer cytology results with conventional smears (10,739 split samples) and concluded that the newer liquid-based cytology system showed a statistically

significant increase in relative sensitivity and no significant difference in relative specificity. The newer liquid-based cytology system showed a 58% reduction in unsatisfactory slides. MonoPrep Pap machine is fully automated and processes 324 samples in 8 hours. The US Food and Drug Administration (FDA) approved this newer liquid-based cytology system in March 2006. However, the report comparing its accuracy with the conventional smear is only anecdotal. Data on histological and human papilloma virus test result correlation and cost effectiveness are still to be reviewed. Without comparison with the 'gold standard' (histology), the clinical accuracy of a system cannot be determined.

In summary, the published literature in this field is very limited and the gold standard histology outcome is not determined in the majority of the trials. Therefore, there is a need to carry out UK based research in order to identify newer, efficient, automated and a cost-effective liquid-based cytology system for the NHS.

### 1.13 Rationale for the present study

There are currently only two liquid-based cytology systems approved for use in cervical cytology in the UK. Therefore, there is lack of competition for liquid-based cytology systems for cervical screening in the UK. This accounts for the high cost per cervical sample in the National Health Service Cervical Screening Programme (NHSCSP) in the UK.

Moreover, the National Institute for Health and Clinical Excellence (NICE) recommended further research into the suitability of alternative slide processing liquid-based instruments for the detection of cervical cancer, and into their possible inclusion in the National Health Service Cervical Screening Programme (NHSCSP). In order for second-generation LBC systems to be introduced in the NHSCSP, they must undergo technical evaluation and assessment of clinical data relating to their sensitivity, specificity and the percentage of inadequate samples must be known (37). Although a few of the newer liquid based cytology systems have been evaluated, there is a lack of robust experimental evidence evaluating the second-generation liquid based cytology systems.

Therefore, the specific aim for this research was to evaluate the second-generation liquid-based cytology systems with the devised protocol and to utilise this protocol in a clinical setting. The results of this evaluation will provide evidence to enable an informed decision on the progression to clinical assessment of second-generation liquid-based cytology systems for cervical screening in the UK.

This study was sponsored by the NHS Purchasing and Supply Agency (PASA), the Centre for Evidence based Purchasing (CEP) on behalf of the National Health Service Cervical Screening Programme. The Manchester

Cytology Centre (MCC) was selected as a site for evaluating the second-generation liquid-based cytology systems. The project was managed by Guildford Medical Device Evaluation Centre (GMEC) on behalf of CEP.

### 1.14 Outline of thesis structure

This thesis has been organised in the following way. The thesis begins with the materials and method, the third part shows the results, the fourth part deals with the discussion and the fifth part summarises the findings with conclusion. Finally, the references and appendices are listed.

# MATERIALS AND METHOD

## 2.1 Materials

Four liquid-based cytology processing systems were initially considered for their suitability in the National Health Service Cervical Screening Programme (NHSCSP). They were

- CellSolutions Europe, CellSolution 120
- Shandon Papspin
- Seroa CYTO-screen
- LGM Liqui-PREP

The Shandon Papspin and the LGM Liqui-PREP are manual methods and were not considered to be suitable for preparing large numbers of samples in the National Health Service Cervical Screening Programme. Seroa CYTO-screen was a semi-automated system, which is no longer available in the UK.

The CellSolution 120$^{TM}$, manufactured by Select Diagnostics, incorporated and supplied by Cell solutions Europe, is an automated liquid based cytology processor. It is computer controlled and has a potential throughput of 120 samples per hour. Moreover, an automated reader for this machine is being prepared. Therefore, CellSolution 120$^{TM}$ was the only product available in the UK, which had the potential to be included in the NHSCSP. This machine has been available in the UK since July 2008. A technical assessment of this product was performed and reliability and ease of use of CellSolution 120$^{TM}$ were determined by our project to be included in the NHSCSP.

## 2.2 Overview of CellSolution 120$^{TM}$ liquid-based cytology system

CellSolution 120$^{TM}$ (Synermed) is CE (conformity mark) marked which, ensures that this product meets European Union consumer safety, health and environmental requirements. CS 120$^{TM}$ is an automated liquid-based cytology processor, which produces bar-coded slides, ready to be stained. CS 120$^{TM}$ is computer controlled. The CS 120$^{TM}$ slides have a thin layer of cells for visual evaluation either manually using a microscope, or a suitable microscopic imaging system unit.

The ethanol preserved CS 120$^{TM}$ cervical sample is concentrated by centrifugation and loaded on the CS 120$^{TM}$ machine to produce microscope slides of approximately equal cellularity. The process is fully automated after the samples have been loaded on the machine and can achieve an optimum throughput of 120 samples per hour.

## 2.3 Describing different methods and rationale for choosing a particular method

Different authors have used various methods to assess the accuracy of second-generation liquid-based cytology systems. They have either compared them to conventional smears, the existing liquid-based cytology systems or to the histology results. The split cervical samples are used for evaluation of newer liquid-based cytology systems.

There has not been any standard protocol to evaluate second-generation liquid-based cytology systems. Therefore, for this study, a protocol was devised to evaluate CellSolution 120$^{TM}$, which was followed in a clinical setting. The devised protocol is shown in figure 9 below.

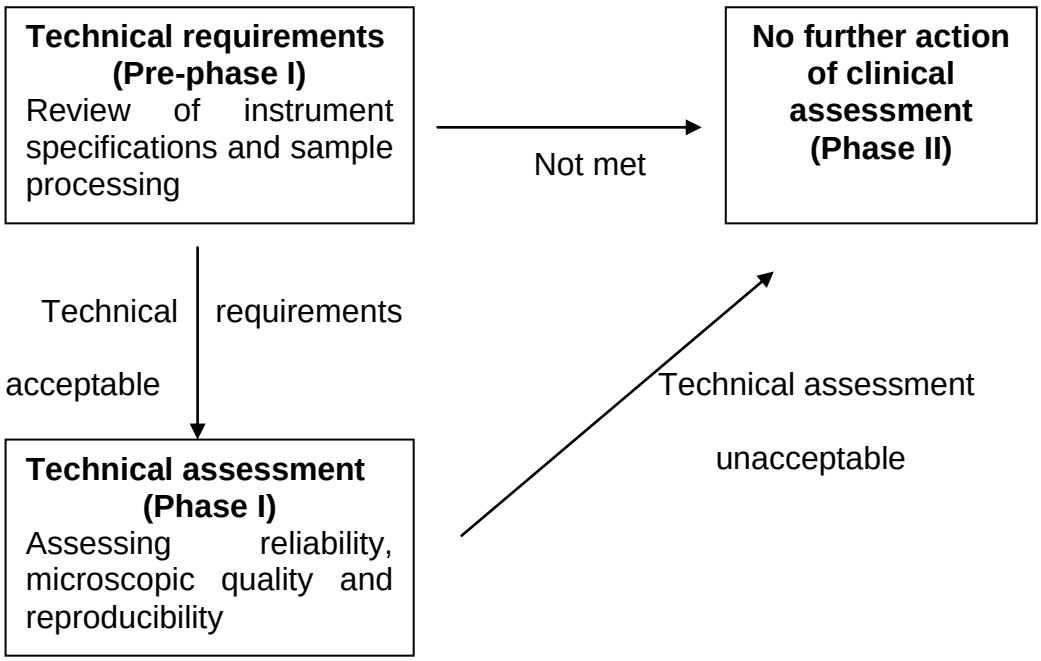



Figure 9: Steps involved in the evaluation protocol of this trial

The second-generation liquid-based cytology system, CellSolution 120$^{TM}$ has not been evaluated in the UK. Therefore, this machine has to be

thoroughly evaluated technically. Technical evaluation will usefully supplement and extend the thorough evaluation of CellSolution 120$^{TM}$ in this project.

The evaluation of CellSolution 120$^{TM}$ was started with pre-phase I, where the technical requirements, namely the instrument specifications and sample processing were reviewed. The evaluation would be carried further to technical assessment (phase I) only if CellSolution 120$^{TM}$ satisfied the technical requirements (pre-phase I). Pre-phase I of the evaluation involved assessing the system to ensure that it met the technical requirements set out by the NHS Cervical Screening Programme (38) (Appendix A). The technical requirements (pre-phase I) to be satisfied by CellSolution 120$^{TM}$ liquid-based cytology system are shown in table 5 below.

Table 5: Technical requirements (pre-phase I) to be satisfied by CellSolution 120$^{TM}$ liquid-based cytology system

| **No** | **Requirements of participating manufacturer/ supplier** | **CellSolution 120$^{TM}$** |
|---|---|---|
| 1 | CE marking (IVDD 98/79/EC) | |
| 2 | Protocol acceptance | |
| 3 | Instrumentation and consumables | |
| 4 | Formal sign-off | |
| 5 | Training and customer support | |
| 6 | Instructions for use and validation | |
| 7 | User list | |
| 8 | Cost information | |
| 9 | Informal / formal comments | |

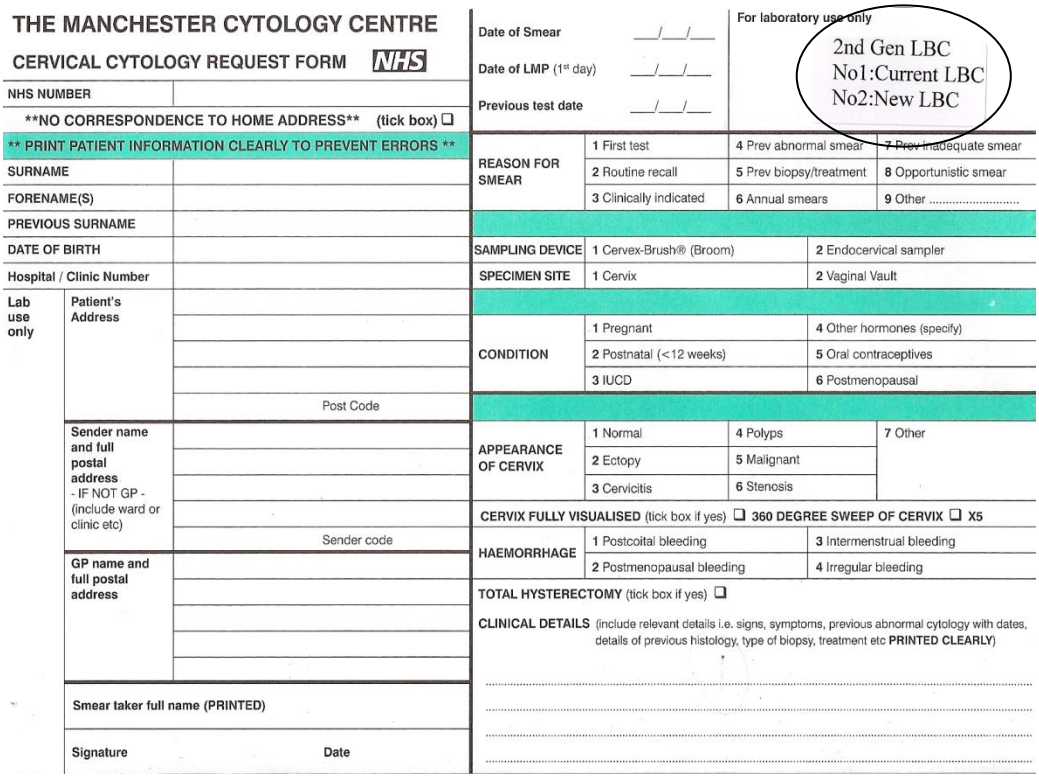
The technical assessment (phase I) assessed the CellSolution 120$^{TM}$ samples for their microscopic quality, reproducibility and reliability. If the CellSolution 120$^{TM}$ system does not meet or satisfy any requirements of phase I of the technical evaluation, there will be no further evaluation of clinical assessment (phase II) as it is proven that machine requirements, sample processing and preparation are not equivalent to the UK standard.

This thesis deals only with pre-phase I and phase I.

## 2.4 Sample size and characteristics

Ethical clearance was sought from National Research Ethics Services (NRES) prior to commencing the study. Central Manchester and Manchester Children's University Hospitals NHS trust was the sponsor for this project.

One hundred electronically randomly assigned samples were used for the technical assessment (phase I) phase. Two samples were collected from each patient at their colposcopy visit. In order to eliminate any collection bias towards any of the liquid-based cytology systems (current and second-generation), the sampling order was randomised. The nurse or doctor took the cervical sample in the order shown in the request form. (Request form showing the sampling order is shown in figure 10 below). This was done, as there is a possibility that the second cervical sample of either the second-generation or the current liquid-based cytology system may be compromised as abnormal cells may have been removed in the first cervical sample.



THE MANCHESTER CYTOLOGY CENTRE

CERVICAL CYTOLOGY REQUEST FORM NHS

NHS NUMBER

**NO CORRESPONDENCE TO HOME ADDRESS** (tick box) ❑

** PRINT PATIENT INFORMATION CLEARLY TO PREVENT ERRORS **

SURNAME

FORENAME(S)

PREVIOUS SURNAME

DATE OF BIRTH

Hospital / Clinic Number

Lab use only

Patient's Address

Post Code

Sender name and full postal address - IF NOT GP - (include ward or clinic etc)

Sender code

GP name and full postal address

Smear taker full name (PRINTED)

Signature Date

Date of Smear ___/___/___

Date of LMP (1st day) ___/___/___

Previous test date ___/___/___

For laboratory use only

2nd Gen LBC
No1:Current LBC
No2:New LBC

REASON FOR SMEAR: 1 First test; 2 Routine recall; 3 Clinically indicated; 4 Prev abnormal smear; 5 Prev biopsy/treatment; 6 Annual smears; 7 Prev inadequate smear; 8 Opportunistic smear; 9 Other .....

SAMPLING DEVICE: 1 Cervex-Brush® (Broom); 2 Endocervical sampler

SPECIMEN SITE: 1 Cervix; 2 Vaginal Vault

CONDITION: 1 Pregnant; 2 Postnatal (<12 weeks); 3 IUCD; 4 Other hormones (specify); 5 Oral contraceptives; 6 Postmenopausal

APPEARANCE OF CERVIX: 1 Normal; 2 Ectopy; 3 Cervicitis; 4 Polyps; 5 Malignant; 6 Stenosis; 7 Other

CERVIX FULLY VISUALISED (tick box if yes) ❑ 360 DEGREE SWEEP OF CERVIX ❑ X5

HAEMORRHAGE: 1 Postcoital bleeding; 2 Postmenopausal bleeding; 3 Intermenstrual bleeding; 4 Irregular bleeding

TOTAL HYSTERECTOMY (tick box if yes) ❑

CLINICAL DETAILS (include relevant details i.e. signs, symptoms, previous abnormal cytology with dates, details of previous histology, type of biopsy, treatment etc PRINTED CLEARLY)

Figure 10: Request form showing the order of sampling to the sample taker

The randomisation details were not revealed to the evaluator and other participants of the trial.

The nurse smear clinic, which is part of the colposcopy clinic was also involved to quicken the completion of the technical assessment (phase I) phase.

A small sample size (n= 100) was chosen because this phase was involved only in assessing the reliability, microscopic quality and reproducibility of CellSolution 120$^{TM}$ samples. Colposcopy samples were targeted for this evaluation, as they are treated as urgent samples, which require quick reporting. Therefore the results of both the liquid-based cytology systems (second-generation and existing) could be quickly obtained for comparison. Also, more varied abnormal cytology categories can be found in colposcopy samples than in the cervical samples sent from general practice clinics.

Therefore, different cytology categories could be assessed for CellSolution 120$^{TM}$ in terms of their reliability and microscopic quality. Moreover, a woman gets time to think about taking part in the trial, as the patient information sheets are sent to her along with the colposcopy appointment letter.

The samples for phase I were obtained from the colposcopy clinic at Salford Royal Hospital and the nurse smear and colposcopy clinic at St. Mary's Hospital, Manchester.

## 2.5 Method in detail

The colposcopy staff at Salford Royal and St. Mary's Hospital, Manchester were informed about the project with a powerpoint presentation and on-going oral communication. The staff of the colposcopy clinics played a vital role in answering participant questions and persuading them to take part in this evaluation. Patient information sheets for phase 1 (Appendix B) were sent to women attending these colposcopy clinics with their appointment letter. At the colposcopy clinics, the women were given time to consult with either a nurse or a doctor regarding the evaluation trial. The participant could either decide to take part in or opt out of the trial. If the woman decided to take part, she needed to sign three copies of the consent form for phase 1 (1 for the researcher, 1 for the patient and 1 for the patient notes) (Appendix C).

The method of processing and evaluating CellSolution 120$^{TM}$ is described below:

### A)- Pre-analytical

1. Each request form was randomly assigned a number by a biomedical scientist (BMS A) prior to sample taking at colposcopy. The BMS A was independent of the analytical process.
2. Two samples for each patient were received at the Manchester Cytology Centre: one for the current LBC system (SurePath$^{TM}$ or ThinPrep$^{TM}$) and the other for CellSolution 120$^{TM}$ (CS 120$^{TM}$) LBC system.
3. After receiving the sample, the laboratory staff took a photocopy of the request form after hiding the randomisation label and kept the CS 120$^{TM}$ sample vial with a copy of the request form aside for the evaluator (E).

4. The original form was shown to the biomedical scientist (BMS A) who entered the randomisation details.
5. The evaluator (E) verified the name, NHS no, date of birth and address (patient details) on the request form and CS 120$^{TM}$ sample vial.
6. The evaluator placed the pre printed CS 120$^{TM}$ barcode labels on the sample vial, request form and primary tube.
7. E processed the CS 120$^{TM}$ primary tube and loaded it on the CS 120$^{TM}$ machine after initial homing of the machine and priming of the tubes on the machine. The samples were processed according to the CS 120$^{TM}$ manual.
8. E kept a record of errors arising and how they were resolved while processing the samples on CS 120$^{TM}$ machine on the datasheet.
9. After the prepared unstained slides were dried, E stained them manually (Appendix D) (initially used the autostainer for staining).

**B)- Analytical**

1. E prepared the datasheets and regularly sent them to CEP. E also sent regular maintenance sheets to CEP. The maintenance sheet is seen in Appendix E and datasheets are attached in Appendix F.
2. E prepared the datasheets for error logging (for each run: error logged, remedial action, outcome and downtime).
3. Quality control (for each run: start and finish time with number of samples processed, macroscopic assessment, repeat and supplemental preparations if any) was carried out by E.
4. Screening (for each slide: macroscopic and microscopic assessment, which consisted of cell presentation, cytolysis, obscuring elements,

nuclear and cytoplasmic staining, 3 dimensionality, cell drift, cell types and stating dyskaryosis if any) was carried out by four individuals- evaluator (E), BMS B and consultant cytopathologists A & B.

The datasheets filled by the evaluator (E) are shown in the table 6 below.

Table 6: Datasheets prepared by the evaluator (E)

| **Quality control sheet** | **Error log sheet** | **Log sheet** | **Ergonomic assessment** |
|---|---|---|---|
| Sample processing | Record of all errors and breakdown | Time required for start and shut down | Emphasis on ease of use |
| Macroscopic appearance | Actions taken and time required to rectify | Maintenance | Operator intervention |
| Overall cellularity | | | Potential for human injury |
| Homogeneity | | | |
| Microscopic appearance | | | |
| Repeat prep? | | | |

5. Evaluator (E) entered her impression for the diagnosis of each slide on the excel sheet and passed the slide to the biomedical scientist (BMS B).

6. BMS B entered her results on the excel sheet and passed it to consultant cytopathologist A with comments regarding preparation of CS 120$^{TM}$ slides, obscuring materials if any, staining quality, adequacy of squamous cells and coverslipping.
7. BMS B also entered the corresponding SurePath$^{TM}$ or ThinPrep$^{TM}$ slide number with their diagnosis after the CS 120$^{TM}$ slide was screened by all the team members.
8. Consultant cytopathologist A entered her results (diagnosis with comments on the preparation, obscuring material, staining, adequacy and coverslipping) on the excel sheet and passed it to consultant cytopathologist B.
9. Consultant cytopathologist B entered her results on the excel sheet (diagnosis with comments on the preparation, obscuring material, staining, adequacy and coverslipping).

**C)- Post-analytical**

1. The final diagnosis for each CS 120$^{TM}$ slide was made with the agreement of more than two team members'.
2. When there was a discrepancy between the results of team members', the result of the majority was finally recorded.
3. In cases (n=2) where there was no majority result between the results of different members of the team, the cases were reviewed by an independent reviewer. The diagnosis of an independent reviewer was taken as a verdict.

4. Also, in cases where there was a discrepancy between the two liquid-based cytology systems, the cases were reviewed by an independent reviewer. These cases were followed up for patient management.
5. Lastly, the CS 120$^{TM}$ slides were archived.
6. After the completion of 100 samples, randomisation details were revealed to E to prevent any bias.

## 2.6 Cervical sampling method for CellSolution 120$^{TM}$

CellSolution 120$^{TM}$ is an automated newer liquid-based cytology system, which produces 120 bar-coded ready to be stained cytology slides in one run. The prepared slides have a thin layer of cells adhering to a defined area of the slide. The machine uses three different fluids for processing. They are:

Water

Glucyte cell adherent

General cytology preservative

The CellSolution 120$^{TM}$ machine is shown in figures 11 and 12 below.

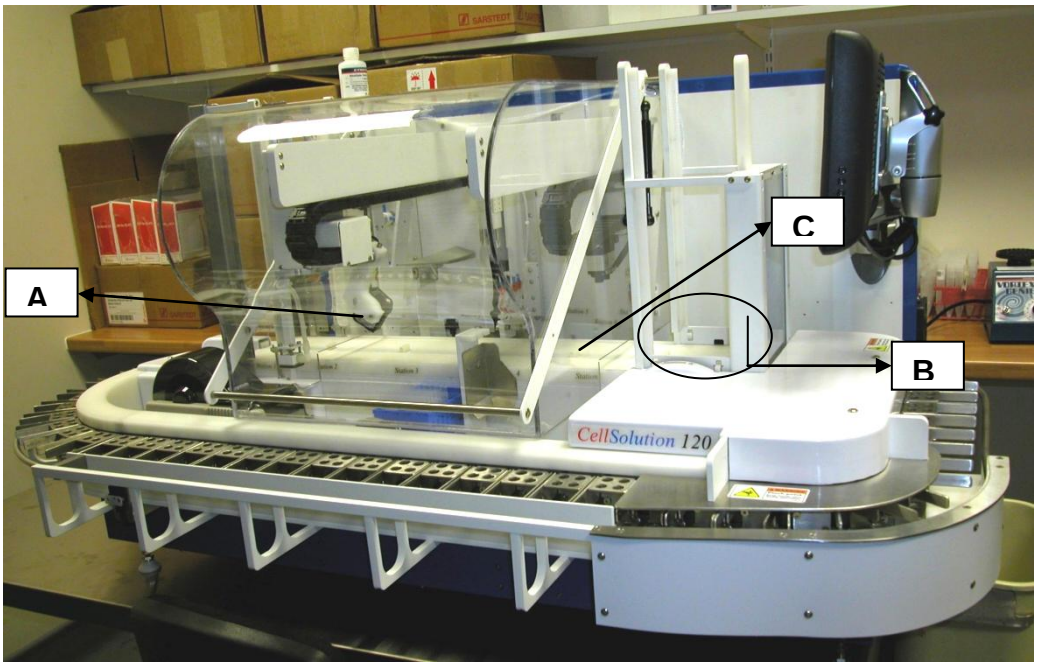



Figure 11: CellSolution 120$^{TM}$ machine, A- barcode scanner, B- slide tray up stacker, C- slide tray conveyer

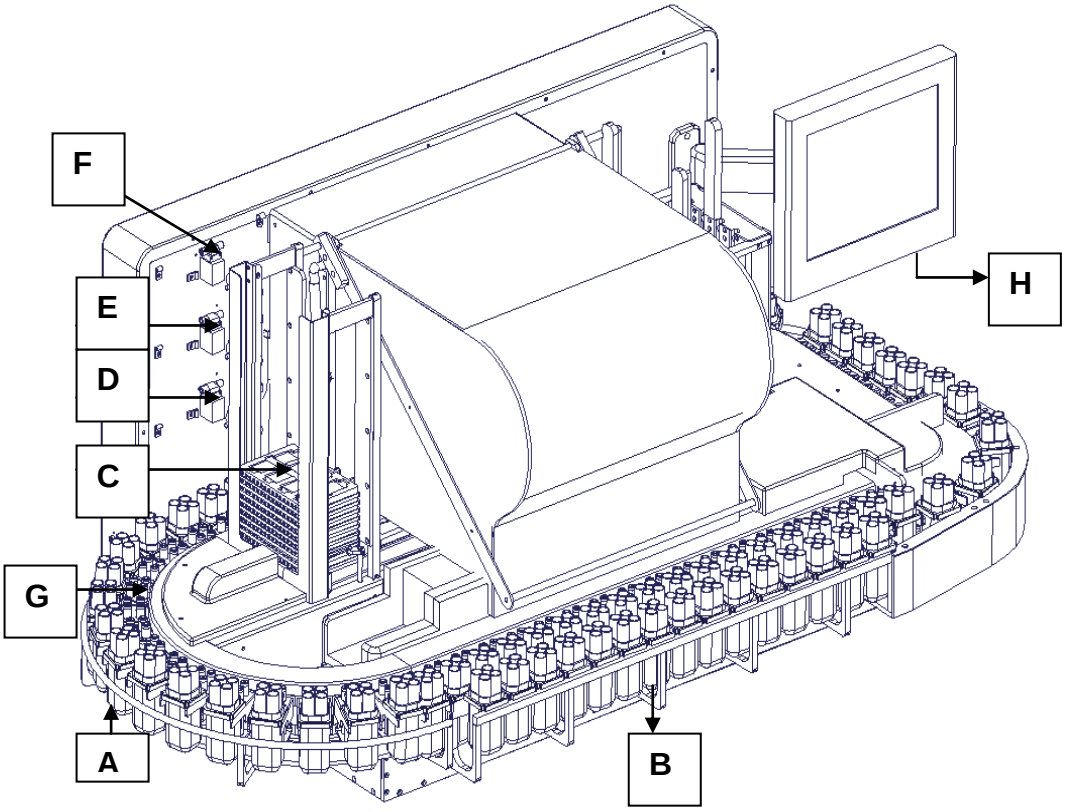



Figure 12: CellSolution 120 machine, A- loaded racks of primary tubes, B- discharge track, C- slide tray down stacker, D- preservative pump, E- glucyte pump, F- water pump, G- secondary tubes corresponding to primary tubes, H- computer screen (this picture is adapted from Cell Solution 120$^{TM}$ user manual)

**Sampling devices and method of collection of a cervical sample:**

An ethanol based preservative vial and brush (sampling devices) for CS 120$^{TM}$ are shown in figure 13 below.

Figure 13: Ethanol based preservative vial and Cervex-Brush® used for CellSolution 120™ cervical sampling

The sampling technique for CS 120™ is similar to the current liquid-based cytology system. It is shown in figure 5 on page 33.

The head of the brush has to be detached and left in the preservative vial. It is shown in figure 14 below.

Figure 14: The head of Cervex-Brush® kept in the CS 120™ preservative vial

## 2.7 CellSolution 120$^{TM}$ operation process

The process requires the CellSolution 120$^{TM}$ device, a centrifuge and a vortexer. The cervical sample is transferred to the primary tube (15 ml) from the preservative vial. The primary tubes are kept in the tube racks, which are provided by the company for centrifugation and use on the CS 120$^{TM}$ device. Firstly, the sample is centrifuged at 2150 revolutions per minute for 10 minutes. Then the sample is decanted, blotted and vortexed so that it can be loaded on the machine after initial homing. The above steps are shown in figure 15 below.

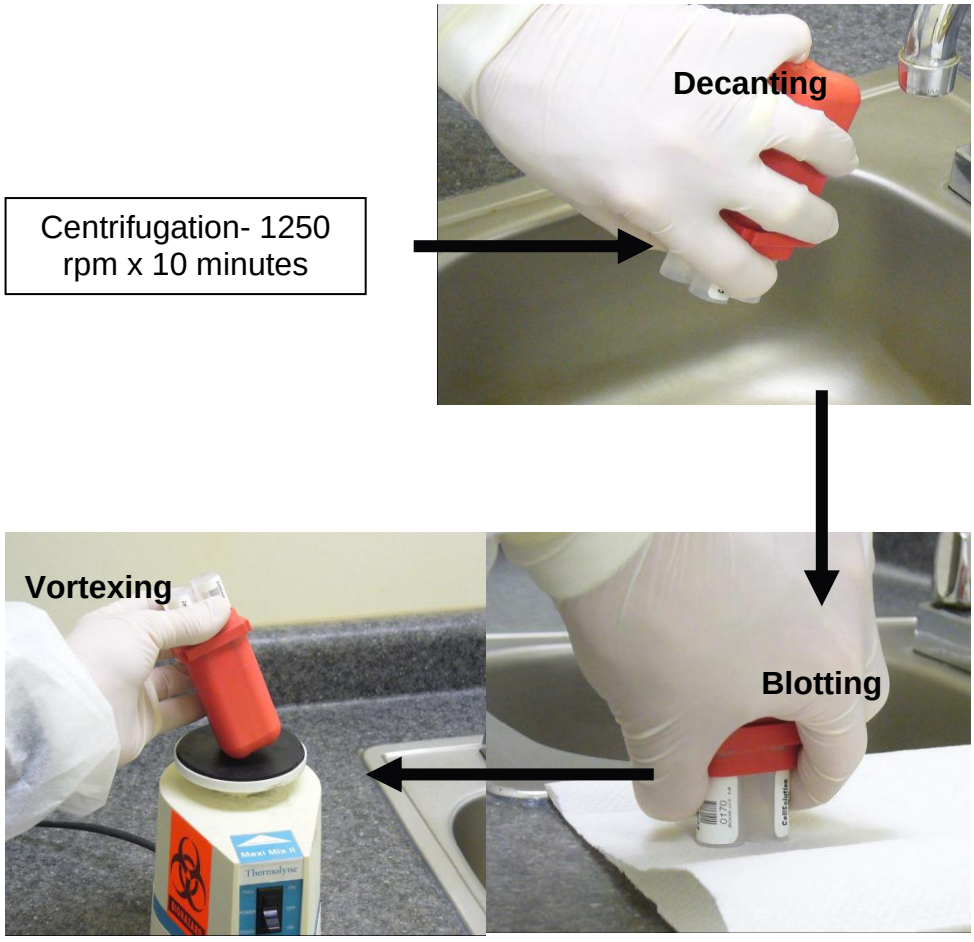



Figure 15: Some of the steps involved prior to loading samples on the CS 120$^{TM}$ machine (Figure adapted from CellSolution 120$^{TM}$ user manual)

The secondary tubes (5 ml) are also loaded along with the primary tubes on the machine. The primary tubes in the tube racks are positioned facing a particular way so that the barcode scanner of the machine is able to read their label. The positioning of primary tubes is shown in figure 16 below.

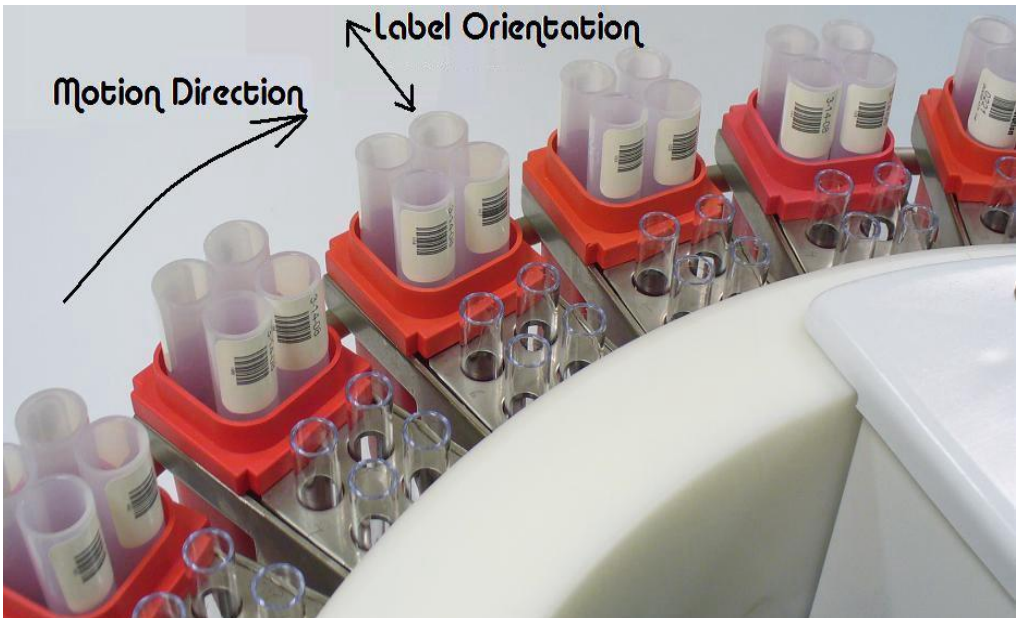



Figure 16: The bar codes on the primary sample tubes should be orientated facing outward relative to the travel direction of the conveyor (Figure adapted from CellSolution 120$^{TM}$ user manual)

Once the machine identifies the sample at station 1, a label corresponding to the primary tube is pasted on the slide by the robotic arm and the height of cell pellet is measured by an ultrasonic sensor at station 2. Tap water is added to the sample for dilution in the primary tube and glucyte is dispensed to the secondary tube by a set of nozzles suspended over the tubes at station 3. The amount of water and glucyte used in the dilution process is based on the number of cells in the original cell pellet. Then, at station 4, two disposable robotically controlled pipette tips and two pipette pumps are used to mix the sample multiple (10) times (an observation) with water in the primary

tube. After mixing, the pumps aspirate a specific volume of the cell mixture and transfer it to the small secondary mixing tube where glucyte has been dispensed. This mixture is aspirated and dispensed several times using the pipette tips to ensure a homogeneous mixture with glucyte. The pump aspirates a specific volume of the solution from the secondary tubes and the robotic arm transfers it to the slide. The robotic arm then disposes of the pipette tips into a collection container. If the device has the conservation mode switched on, then after dispensing the sample on the slide, the robotic arm preserves the remaining cytology sample into the primary tube and then disposes of the pipette tips.

At station 6, the prepared slides are dried in an in-built air-drying chamber for forty minutes. Preservative is added to the primary tubes at station 7 to store the cervical sample. Later on, the slides are stained with the set-up manual staining system (Appendix D). Initially, the staining was done using the Leica Autostainer XL, but then had to shift to the manual staining system as Leica Autostainer XL is not the actual system, which is to be used later on with the CS 120$^{TM}$ slides. Eventually, an automated slide staining and cover slip machine will have to be used if the CS 120$^{TM}$ is approved for use in the NHSCSP.

The above is an overview of the CellSolution 120$^{TM}$ operation process. The positions of the slide at different stations, primary and secondary tubes are clearly shown on the computer screen in figure 17 below.

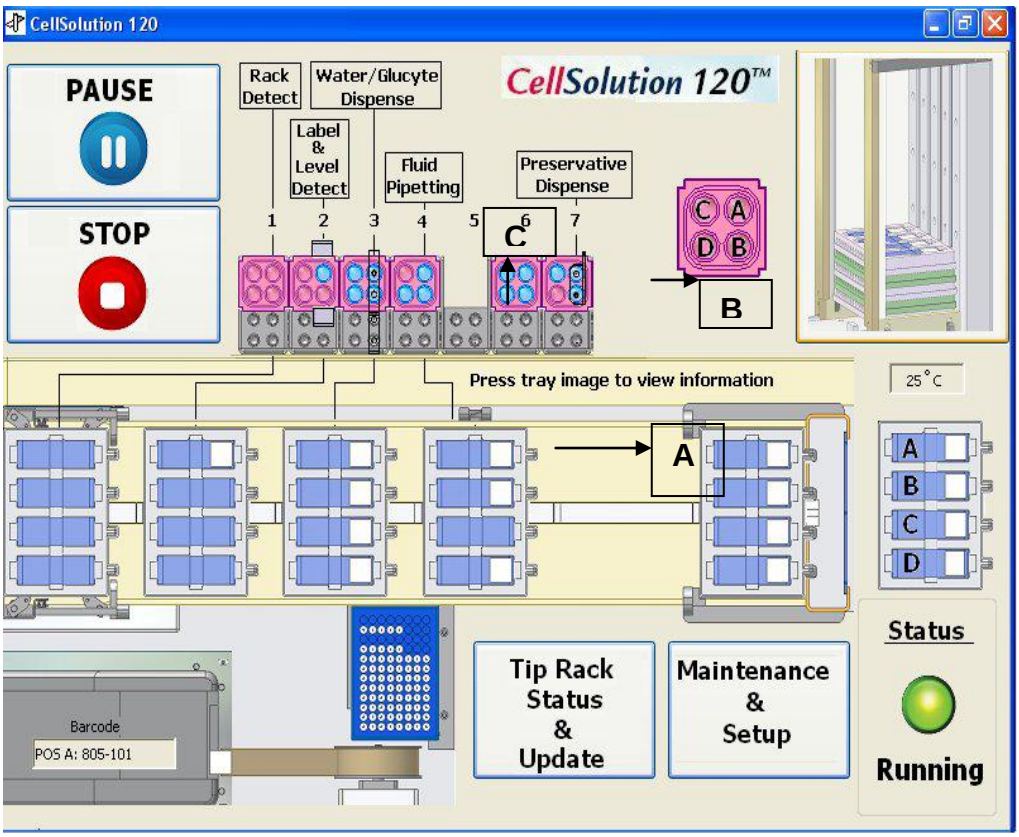



Figure 17: Computer screen showing positions of prepared slides (A), primary (B) and secondary tubes (C) (Picture adapted from CellSolution 120™ user manual)

A record of the processed samples, the amounts of water and glucyte used, the amount of sample transferred to the slide, the start and end times and errors are maintained on the computer. Moreover, these data are easy to retrieve and store in a memory stick. A sample record is shown in tables 7 and 8 below. The tables are adapted from CellSolution 120™ user manual.

Table 7: CellSolution 120™ operational log file

| **CellSolution 120™ Operation Log File**<br>**Software Rev. 1.0**<br>**Unit 1002**<br>**2008-12-17** | | |
|---|---|---|
| **Date** | **Time** | **Process** |
| 12/17/08 | 15:29:59 | Program Started |
| 12/17/08 | 15:46:16 | Unit Start-up Sequence completed by ww |
| 12/17/08 | 15:46:16 | Glucyte Lot No 050805, expire 05.05.2010 |
| 12/17/08 | 15:46:16 | Sample Conservation Mode: ON |
| 12/17/08 | 15:46:16 | Cellularity Adjustment: 1.0 |
| 12/17/08 | 15:46:16 | Preservative Addition: 1500 |
| 12/17/08 | 15:49:01 | Error 610-3: Ultrasonic sensor reading out of range |
| 12/17/08 | 16:02:35 | Process Terminated |
| 12/17/08 | 16:07:38 | Sample Count = 684 |
| 12/17/08 | 16:07:38 | Exit to Windows |

Table 8: CellSolution 120$^{TM}$ sample log file

| **CellSolution 120$^{TM}$ Sample Log File**<br>**Unit 1002**<br>**2008-12-01** | | | | | | |
|---|---|---|---|---|---|---|
| **Scan Date** | **Scan Time** | **ID** | **Pellet Vol** | **Water** | **Glucyte** | **Transfer** |
| 2008-12-01 | 09:58 AM | 038 | 65.21 | 177.87 | 200.00 | 60.00 |
| 2008-12-01 | 09:58 AM | 148 | 51.63 | 140.84 | 200.00 | 60.00 |
| 2008-12-01 | 09:59 AM | 037 | 71.14 | 194.06 | 200.00 | 60.00 |
| 2008-12-01 | 10:00 AM | 036 | 701.27 | 1912.94 | 200.00 | 60.00 |
| 2008-12-01 | 10:00 AM | 033 | 94.99 | 259.10 | 200.00 | 60.00 |

The amount of water per sample varies between 100 and 1000ul and the glucyte amount is 200ul in nearly all the samples. The software of the machine uses an algorithm to adjust the amount of cells applied to the slide (cellularity) by varying the amount of water, glucyte, and transfer volumes for each individual sample. This dilution process is based on an approximation of the number of cells starting in the pelletized sample. The sample dilution algorithm is shown in Appendix G.

Different aspects of the maintenance of the CS 120™ device take place on a daily, weekly and semi-annual basis (Appendix E).

**Summary of CellSolution 120™ operating process**

Slide Handling

Tube Handling

Specimen Identification

Specimen Volume Detection

Specimen Dilution

Specimen Mixing and Transfer

Specimen Application to Slide

Specimen Drying

## 2.8 Indicating problems in this evaluation

In this complete evaluation, the major difficulty was in obtaining the samples. The women were not keen to take part as their sample is being evaluated rather than being tested on the standard system. They have a perception that if the machine does not pick up the abnormal cells, it will lead them to pain and trouble. And also since many trials requiring cervical samples are taking place at St. Mary's Hospital to make the National Health Service Cervical Screening Programme more robust and effective, samples were not easily available for this evaluation. Therefore, National Research Ethics Services (NRES) approval was sought in January 2009 for obtaining samples from the nurse smear clinic at St. Mary's Hospital along with the colposcopy samples for the completion of the technical assessment (phase I). Moreover, the full capacity of CellSolution 120$^{TM}$ liquid-based cytology system (120 samples) was not tested. One hundred and twenty SurePath$^{TM}$ samples were run on this machine due to unavailability of CS 120$^{TM}$ samples. The experience of running 120 SurePath$^{TM}$ samples has been valuable.

# RESULTS

### 3.1 Results of technical requirements (pre-phase I) of CellSolution 120™ liquid-based cytology system

The results of technical requirements of CellSolution 120™ liquid-based cytology system are shown in table 9 below.

Table 9: Results of technical requirements (pre-phase I) of CellSolution 120™ liquid-based cytology system

| No | Requirements of participating manufacturer/ supplier | CellSolution 120™ |
|---|---|---|
| 1 | CE marking (IVDD 98/79/EC) | √ |
| 2 | Protocol acceptance | √ |
| 3 | Instrumentation and consumables | √ |
| 4 | Formal sign-off | √ |
| 5 | Training and customer support | √ |
| 6 | Instructions for use and validation information | √ |
| 7 | User list | √ |
| 8 | Cost information | |
| 9 | Informal/formal comments | √ |

The cost of Cell Solution 120™ machine has not been disclosed to the team members and the participants. However, the cost can be made available through the distributors of CellSolution Europe after the clinical assessment (phase II) is completed. The remaining technical requirements of pre-phase I were satisfied by the CellSolution 120™ liquid-based cytology system

## 3.2 Results of technical assessment (phase I) of CellSolution 120™ liquid-based cytology system

### 3.2a Ergonomic assessment results

Operating the CellSolution 120™ device is easy. The presentation of the cervical samples tested on CellSolution 120™ is shown in figure 18 below.

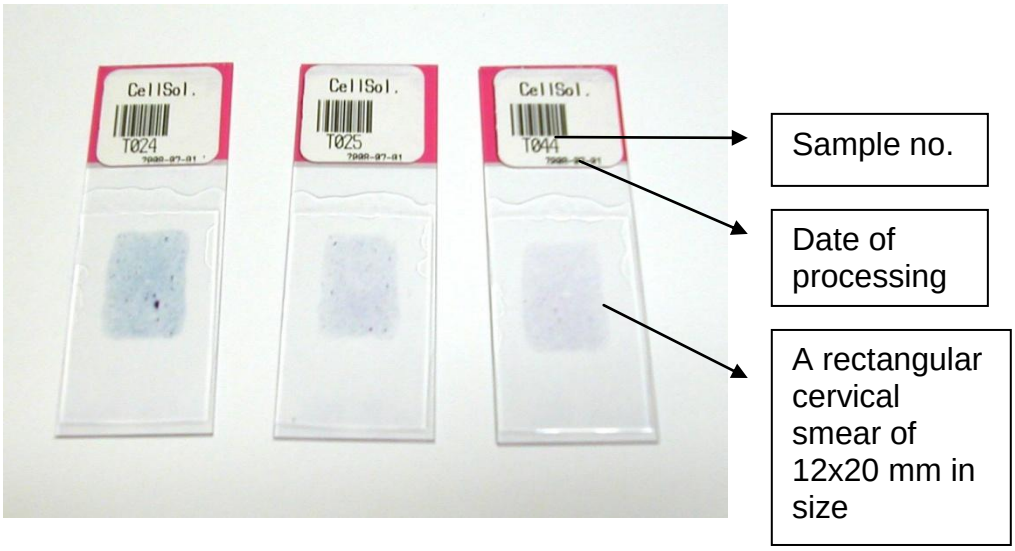



Figure 18: End result of samples processed on CellSolution 120™ machine

An ergonomic assessment, which was part of the technical specifications verification, was carried out on the CellSolution 120™ liquid-based cytology system. Different aspects of the operation of the instrument were assessed with particular emphasis on ease of use, the level of operator intervention and the potential for human error or injury. The observations are shown in table 10 below.

Table 10: Results of ergonomic assessment of CellSolution 120™ liquid-based cytology system

| **CellSolution 120™ operational aspects** | **Ease of use** | **Potential for human error or injury** |
|---|---|---|
| Physical access to system for loading samples, reagents, maintenance and trouble shooting | Easy | Need to be careful while loading samples |
| **General aspects of CS 120™ software use** | | |
| **CellSolution 120™ operational aspects** | **Ease of use** | **Potential for human error or injury** |
| Loading consumables and reviewing on-board stock | Easy | No |
| Preparing and loading samples | Labour intensive, need to have a large capacity centrifuge. Need to have a specific label position on the primary tube, so that barcode scanner can scan | Need to be careful while transferring the sample from the preservative vial to the primary tube |
| Preparing and loading reagents | Easy | Need to be careful and insert the respective pump tubes into the respective reagents |

Table 10: Results of ergonomic assessment of CellSolution 120™ liquid-based cytology system (continued)

| **CellSolution 120™ operational aspects** | **Ease of use** | **Potential for human error or injury** |
|---|---|---|
| Monitoring on board reagent volumes and expiry dates | Easy | No |
| Monitoring sample progress and expected completion time | Good, though takes more time to dry than the 40 minutes, which is stated in the user manual | No |
| Sample loading and unloading | Easy | Need to be careful with the fingers while loading and unloading (there is a notice of caution on the machine) |
| Sample identification | Ok if primary tubes are placed in a particular direction | No re-verification of label is done by the scanner |

Table 10: Results of ergonomic assessment of CellSolution 120™ liquid-based cytology system (continued)

| **CellSolution 120™ operational aspects** | **Ease of use** | **Potential for human error or injury** |
|---|---|---|
| Starting up and shutting down the instrument, time and instances required | Quick | While homing the instrument, need to be careful as it is easy to forget to pick up the small containers kept for collection of primed fluids |
| Result reporting and reviewing, including printing options if not connected to Laboratory Information System | Memory stick can be used to retrieve the data (monthly sample log and operational log) | No |
| Performing maintenance tasks | Easy and well documented except for the tube buckets | No |

Table 10: Results of ergonomic assessment of CellSolution 120$^{TM}$ liquid-based cytology system (continued)

| **CellSolution 120$^{TM}$ operational aspects** | **Ease of use** | **Potential for human error or injury** |
|---|---|---|
| Interpretation of error messages | Easy to understand and follow the instructions | No |
| Quality of trouble shooting, information provided by the manufacturer | Adequate, however, need to hear noise when an error has occured | |
| Sample tracking system if available | Label number can be seen on the screen. The “ABCD” box, which gives information about the slides and their respective tubes is useful | No |
| Compatibility with slide stainers and coverslips if not included in the staining system | Easy, tried on Leica Autostainer XL and Leica robotic coverslipper CV5030 | No |

Overall, the CellSolution 120$^{TM}$ liquid-based cytology system was successful in ergonomic assessment. The preparation of the samples was

lengthy. However, after the samples have been loaded, the machine completed the work unless an error occurred while processing. Overall, the CS 120$^{TM}$ machine was user friendly.

### 3.2b Macroscopic assessment of the slides prepared on CellSolution 120$^{TM}$ liquid-based cytology system

The CS 120$^{TM}$ produces a rectangular smear with an average size of 12x20 cm. Two smears are shown in figure 19 below.


Figure 19: Macroscopic appearance of the end product, rectangular smears (12x20 cm) produced by CellSolution 120$^{TM}$ liquid-based cytology system

### 3.2c Microscopic assessment of the slides prepared on CellSolution 120™ liquid-based cytology system

The cells look similar to those seen in the SurePath™ or ThinPrep™ liquid-based cytology preparations. This can be seen in the figures below, which show intermediate squamous cells, endometrial cells and endocervical cells.

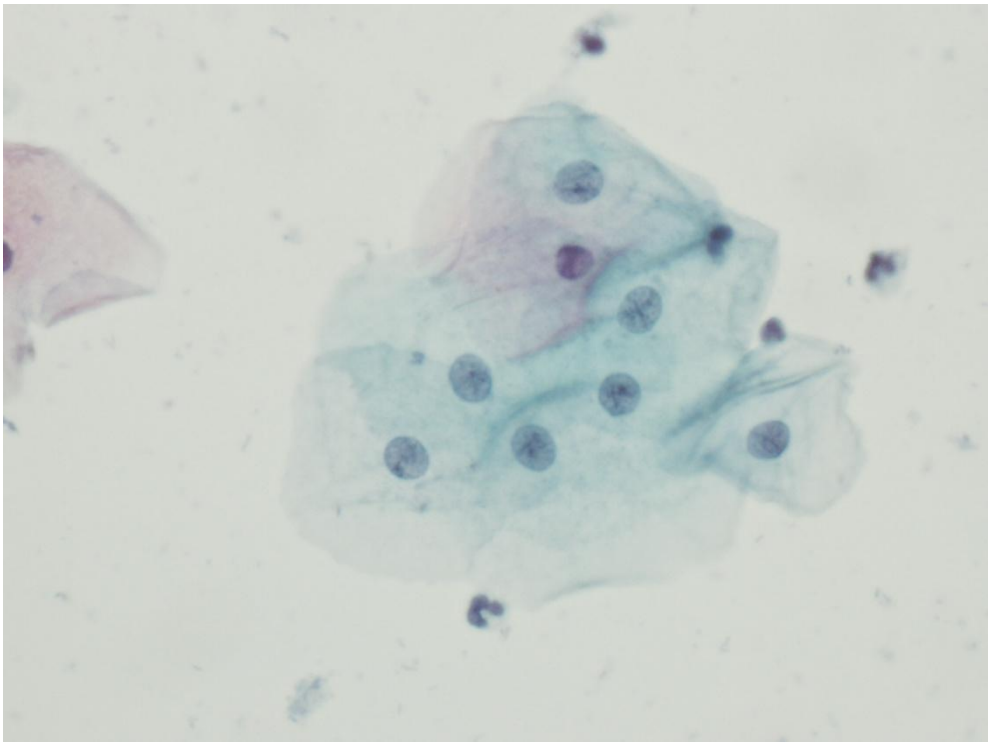


Figure 20: Clumped intermediate squamous cells with nuclear grooves (60x)

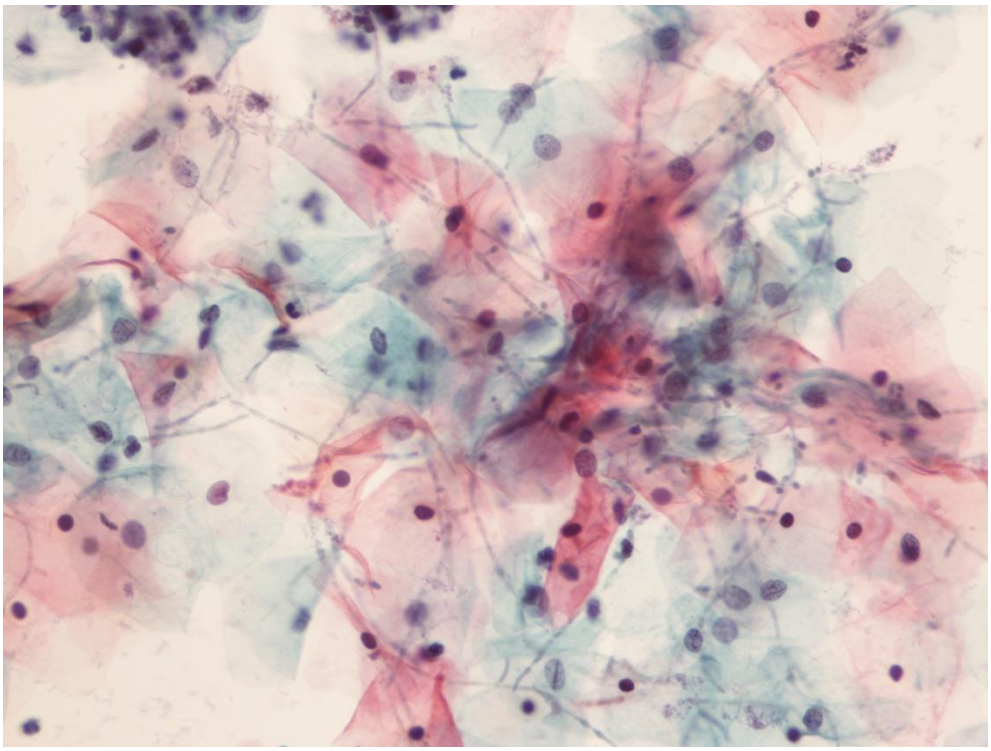


Figure 21: Negative cytology with candida (40x)

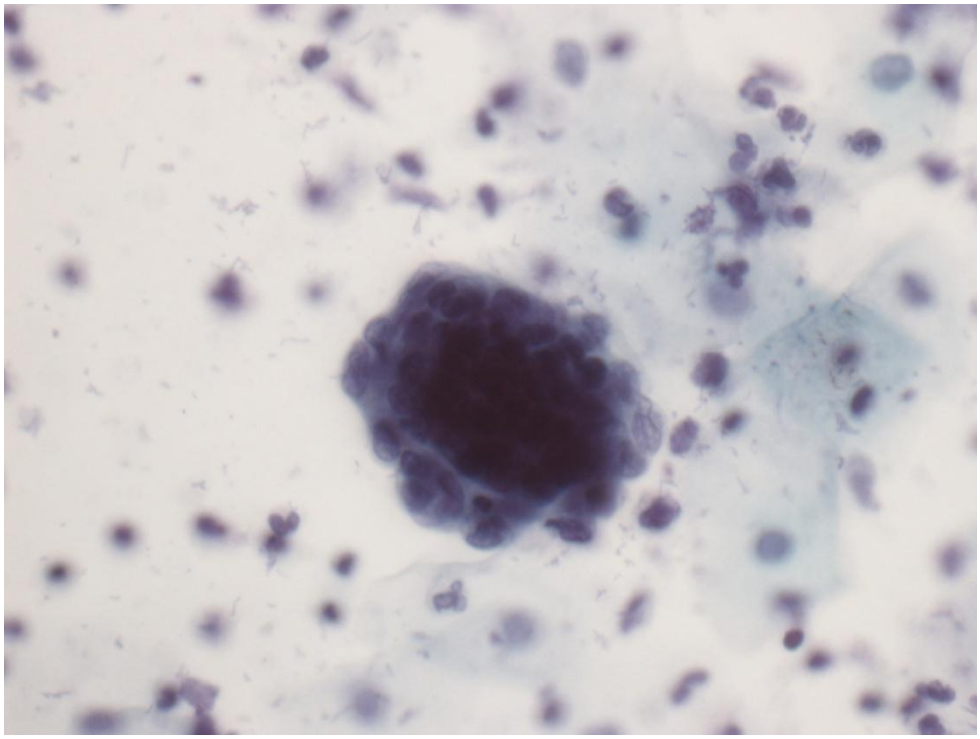


Figure 22: Top-hat arrangement of endometrial cells (60x)

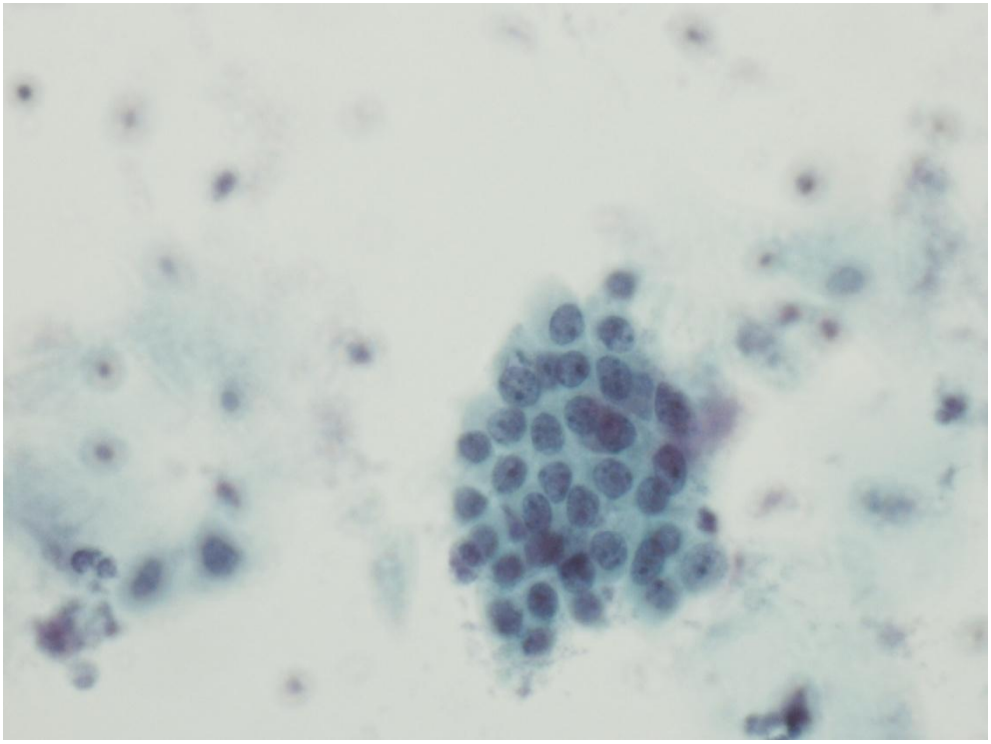


Figure 23: Honeycomb sheet of endocervical cells (60x)

Moreover, the dyskaryotic cells in CellSolution 120$^{TM}$ preparations are similar to those seen either in SurePath$^{TM}$ or ThinPrep$^{TM}$ preparations. Koilocytes, mild dyskaryosis and a hyper chromatic crowded cell group of high-grade dyskaryosis are shown below.

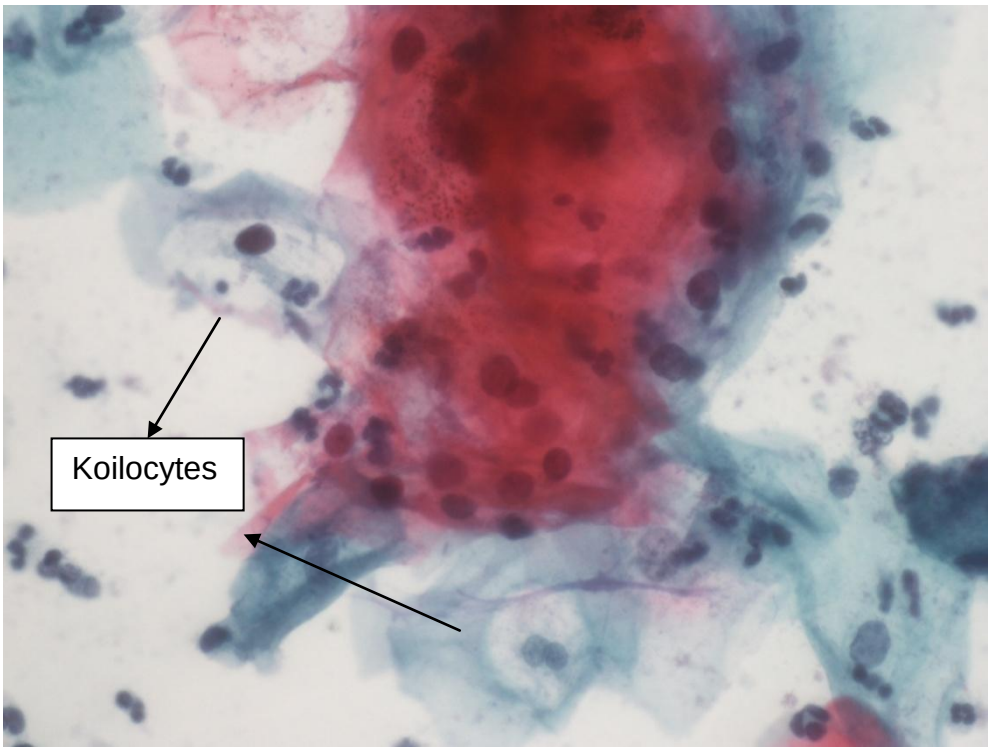



Figure 24: Koilocytes with pencil thick cell border, clear halo beneath the cell membrane and nuclei with grainy nuclear chromatin (60x)

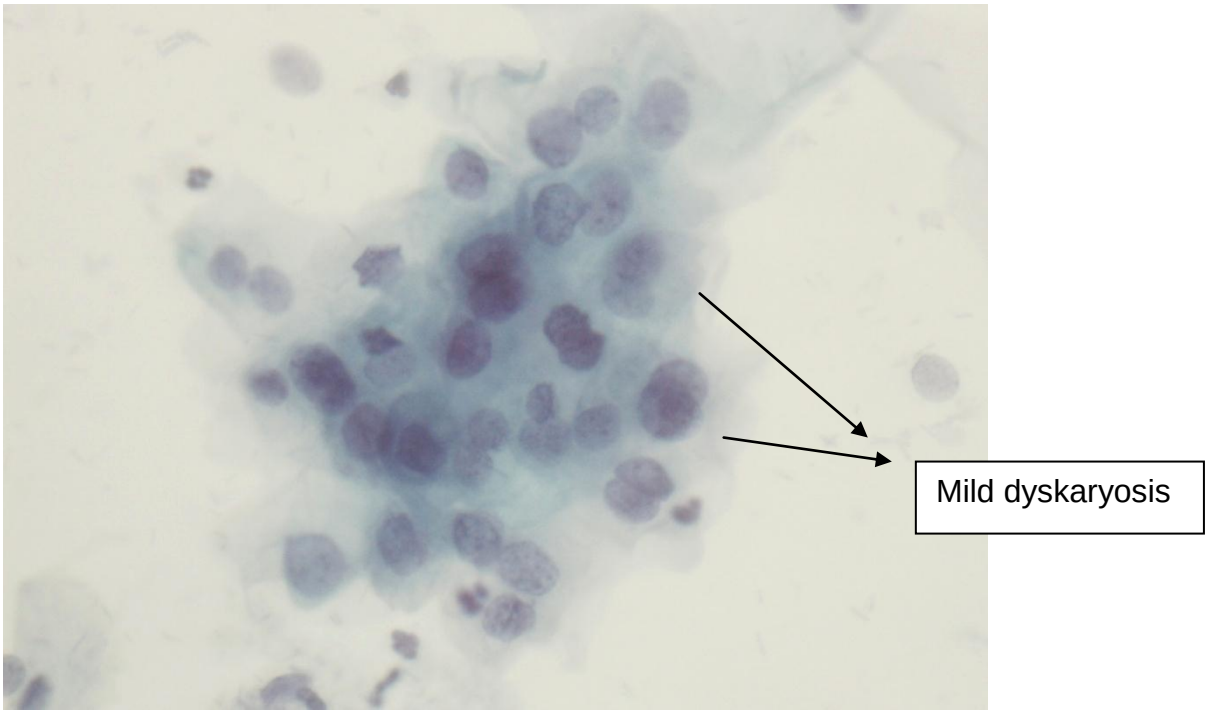



Figure 25: A group of cells showing mild dyskaryosis where the abnormal nuclei are occupying more than one-third, but, less than half the total cell area (60x)

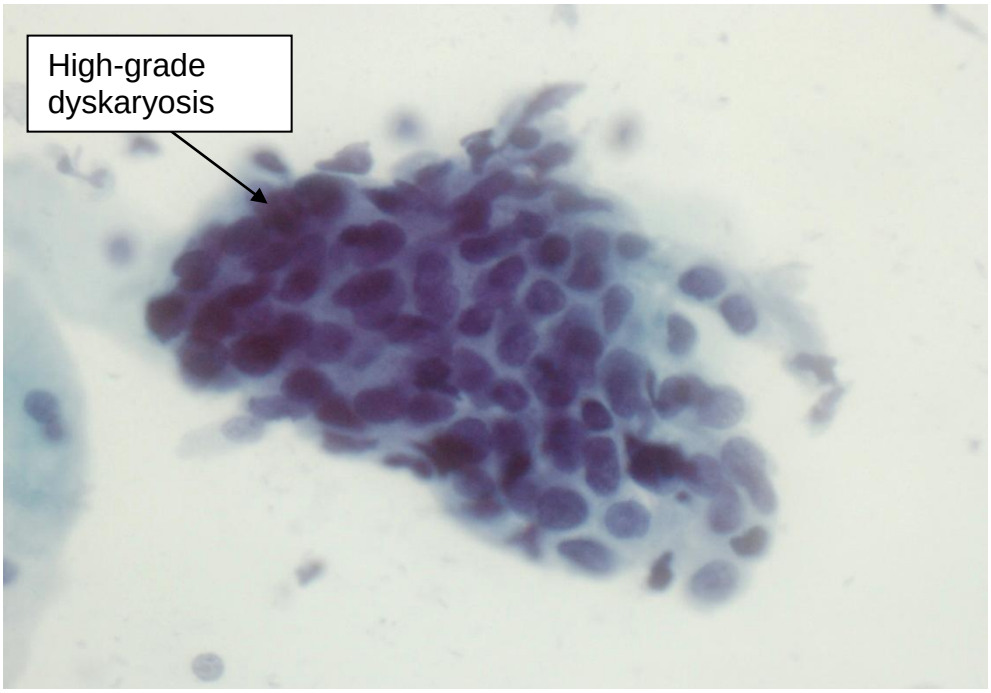



Figure 26: Hyperchromatic crowded cell group showing moderate to severe dyskaryosis (60x)

### 3.3 Problems encountered during the evaluation of CellSolution 120™ liquid-based cytology system

One hundred cervical samples were collected from colposcopy departments at St. Mary's Hospital and Salford Royal Hospital and from the nurse-smear clinic at St. Mary's Hospital for the evaluation of CellSolution 120™ liquid-based cytology system. Two cervical samples were collected from each woman, i.e. one for the current liquid-based cytology system and the second sample for CellSolution 120™. The evaluator (E), biomedical scientist (BMS B) and two consultant cytopathologists (A & B) screened CellSolution 120™ (CS 120™) slides. The evaluation of CS 120™ slides is documented on the excel sheet and datasheets.

During the processing of the cervical samples on CellSolution 120™, a few problems were observed. The manufacturer of CS 120™ was contacted and this resulted in certain modifications to the CS 120™ machine. The problems were:

1. **Printer:**

   The robotic arm was unable to pick up some labels and paste them on the slides. A slide without a label is shown in figure 27 below. This was encountered initially, as there was no support to the label reel and the labels slipped off the printer head.

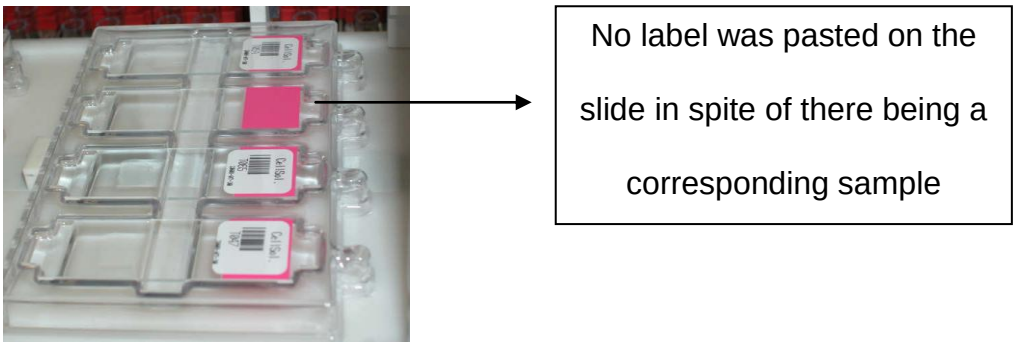



Figure 27: No label on the CS 120$^{TM}$ slide as the robotic arm failed to pick it up from the printer head

2. **Macroscopic quality of slides:**

Frequently, gaps were seen on the prepared slides. The figure below shows a gap on a smear.

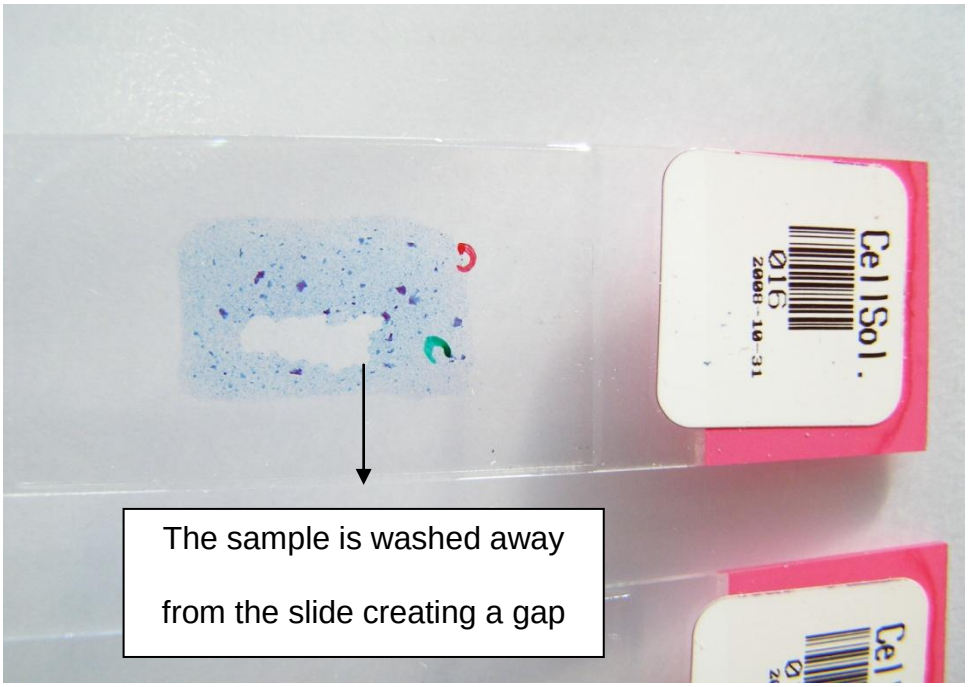



Figure 28: CS 120$^{TM}$ sample showing a big gap

3. **Microscopic quality of slides:**

Variable staining was observed. A sample with such staining is shown in figure 29 below.

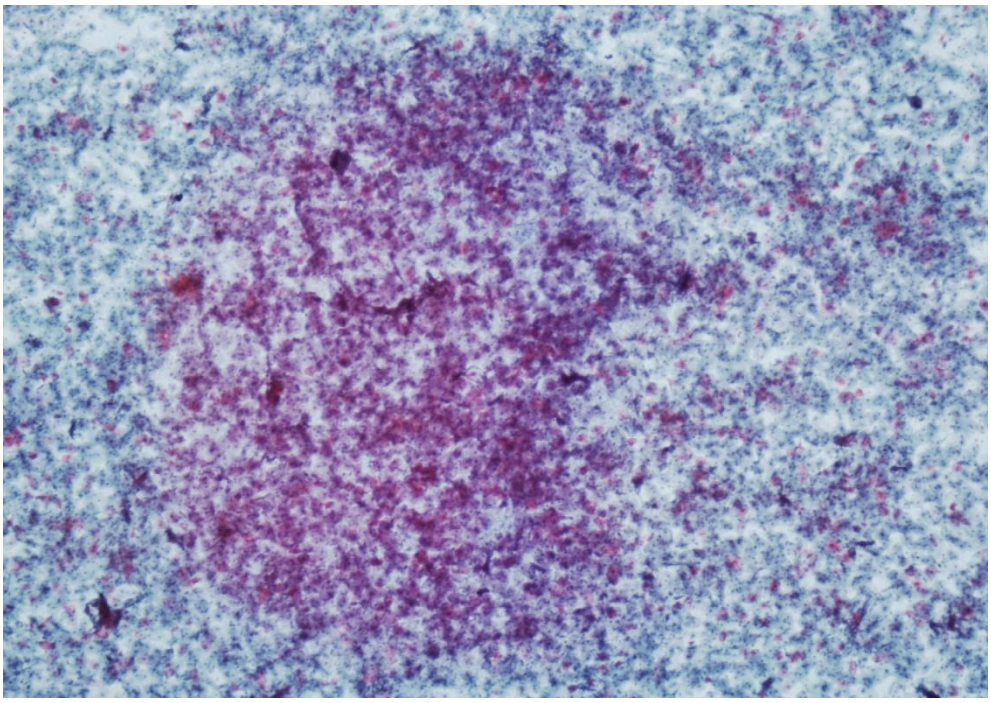


Figure 29: Variable staining on a CS 120$^{TM}$ slide (4x)

Obscuring elements like polymorphs, bacilli and background material were seen on the cells, which made assessment of the CellSolution 120$^{TM}$ slides very difficult. Due to this, thirteen CS 120$^{TM}$ samples out of 65 samples (20%) were reported as inadequate, while only four SP or TP samples out of 65 samples (6.15%) were reported as inadequate. The inadequacy rate was high as compared to existing liquid-based cytology systems. A comparison of CS 120$^{TM}$ samples with the SP/TP samples is shown in figure 30 below for the first sixty-five cervical samples.

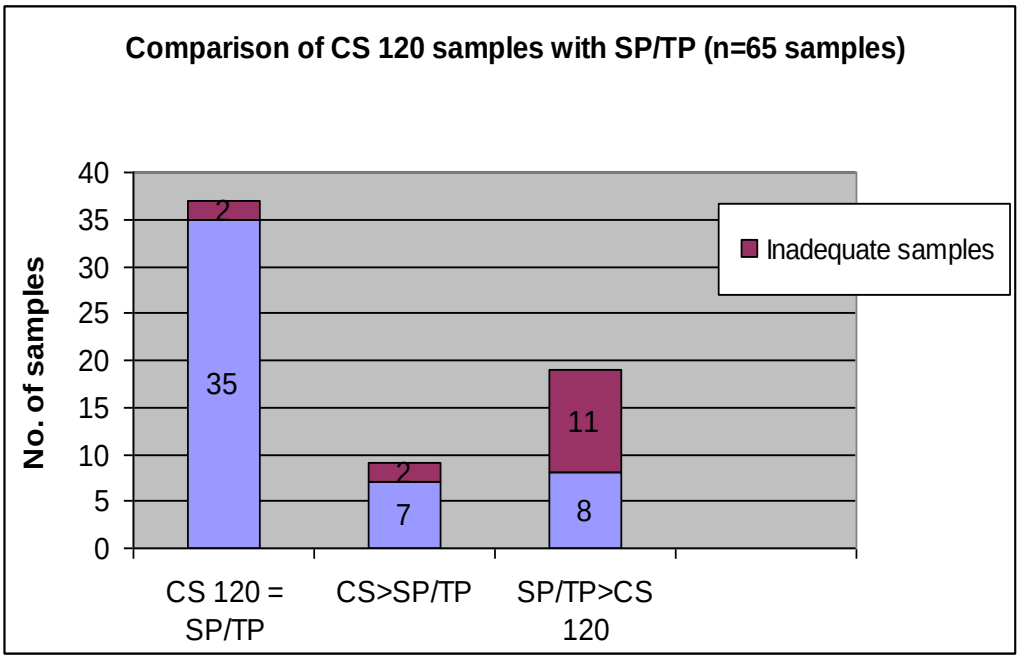



Figure 30: Comparison of CellSolution 120$^{TM}$ cervical samples with SurePath$^{TM}$ or ThinPrep$^{TM}$ samples

Due to the high inadequacy rate, eleven CS 120$^{TM}$ cervical samples were compared with their corresponding SurePath$^{TM}$ or ThinPrep$^{TM}$ samples. Details on cell preservation, cytoplasm and nuclear staining, background material and obscuring elements were evaluated between two liquid-based cytology systems (current and newer) by the evaluator (E) and consultant cytopathologist B. The comparison is shown in table 11 below.

Table 11: Comparison of CellSolution 120™ (CS 120) samples with corresponding SurePath™ (SP) / ThinPrep™ (TP) samples

| No. | Sample No. | CS/SP/TP | 1st/2nd sample | Diagnosis | Cell preservation | Cytoplasm staining Blue/green | Cytoplasm staining Pink/Orange | Nuclear staining | Background material | Obscuring |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 055 | CS | 2nd | Severe dyskaryosis | Poor | Poor | Good | Acceptable | Bacteria +++ | +++ |
| | 1 | SP | 1st | Borderline nuclear changes | Good | Good | Good | Good | Bacteria ++ | + |
| B | 056 | CS | 1st | Negative | Moderate | Good | Good | Good | Polys ++ | ++ |
| | 2 | SP | 2nd | Negative | Good | Good | Good | Good | Polys + | ---- |
| C | 057 | CS | 2nd | Severe dyskaryosis | Poor | Poor | Good | Acceptable | Bacteria & polys +++ | +++ |
| | 3 | SP | 1st | Severe dyskaryosis | Good | Good | Good | Good | Bacteria & polys+ | + |
| D | 058 | CS | 2nd | Negative | Good | Poor | Good | Acceptable | Bacteria + | --- |
| | 4 | SP | 1st | Negative | Good | Good | Good | Good | Very little | --- |
| E | 059 | CS | 1st | Negative | Poor | Poor | Acceptable | Acceptable | Bacteria +++ | +++ |
| | 5 | SP | 2nd | Negative | Good | Good | Good | Good | Very little | --- |
| F | 060 | CS | 1st | Negative | Good | Good | Good | Good | Very little | --- |
| | 6 | SP | 2nd | Negative | Good | Acceptable | Good | Good | Very little | --- |
| G | 061 | CS | 2nd | Mild dyskaryosis | Good | Acceptable | Good | Acceptable | Bacteria + | --- |
| | 7 | SP | 1st | Mild dyskaryosis | Very good | Very good | Very good | Good | --- | --- |
| H | 062 | CS | 2nd | Borderline nuclear changes | Moderate | Acceptable | Acceptable | Acceptable | Bacteria & debris +++ | +++ |
| | 8 | SP | 1st | | | | | | | |
| I | 063 | CS | 2nd | Mild dyskaryosis | Good | Acceptable | Good | Acceptable | Bacteria +++ | +++ |
| | 9 | SP | 1st | Mild dyskaryosis | Good | Acceptable | Good | Good | Bacteria ++ | --- |

Table 11: Comparison of Cell Solution 120™ (CS 120) samples with corresponding SurePath™ (SP) / ThinPrep™ (TP) samples (continued)

| No. | Sample No. | CS/SP/TP | 1st/2nd sample | Diagnosis | Cell preservation | Cytoplasm staining Blue/green | Cytoplasm staining Pink/Orange | Nuclear staining | Background material | Obscuring |
|---|---|---|---|---|---|---|---|---|---|---|
| J | 064 | CS | 2nd | Borderline nuclear changes | Good | Poor | Poor | Acceptable | Polys + | --- |
| | 10 | SP | 1st | Borderline nuclear changes | Good | Acceptable | Acceptable | Acceptable | Very little | --- |
| K | 065 | CS | 1st | Negative | Good | Good | Good | Good | Polys +++ | +++ |
| | 11 | TP | 2nd | Negative | Good | Poor | Acceptable | Good | Polys + | --- |

The macroscopic and microscopic appearances of one of the obscured samples are shown in figure 31 and figure 32 respectively.


Figure 31: Macroscopic appearance of the CS 120$^{TM}$ sample obscured by varied elements

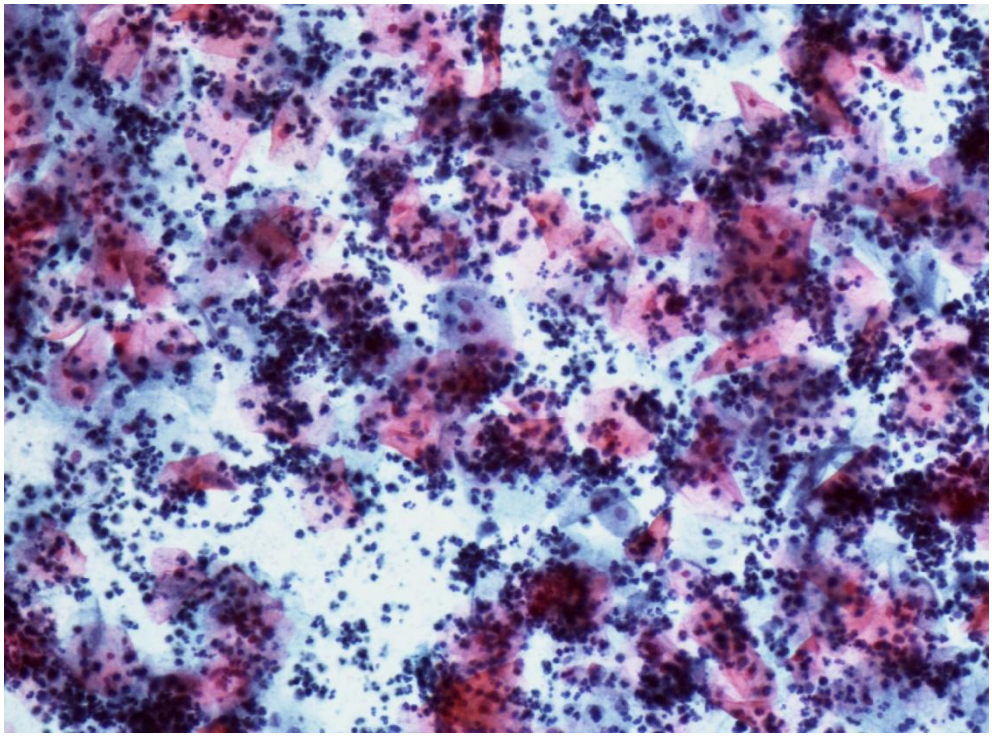


Figure 32: Squamous cells are obscured by polymorphs and bacilli (20x) in CS 120™ sample, which resulted in an inadequate report

One of the CS 120™ samples is compared with the ThinPrep™ sample. Their microscopy result is shown in figures 33 and 34 respectively below.

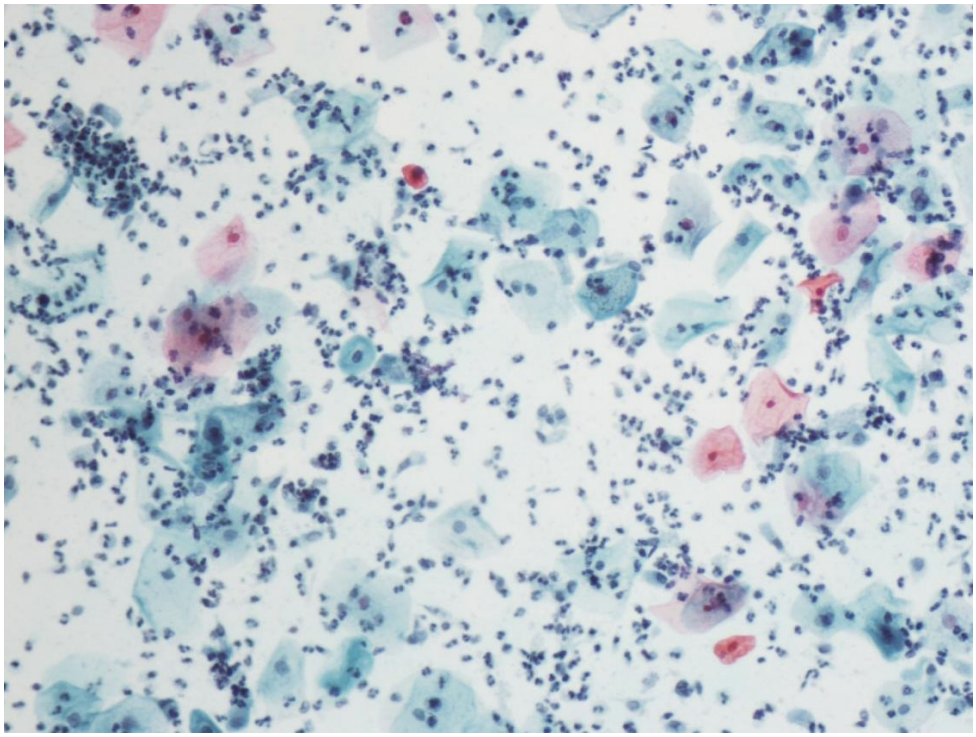


Figure 33: Squamous cells are obscured by polymorphs and bacilli (20x) in CS 120™ sample, which resulted in an inadequate report

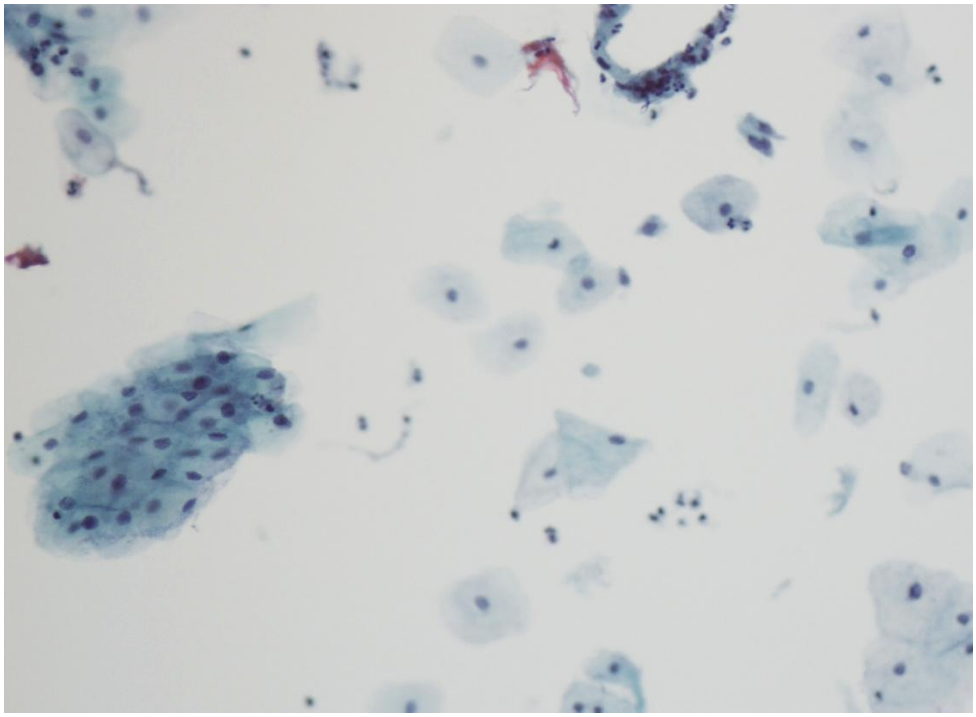


Figure 34: ThinPrep™ sample corresponding to above CS 120™ sample showing well-dispersed and unobscured squamous cells (20x)

The CellSolution 120$^{TM}$ slides with obscured appearances such as those shown above were not acceptable. As a result, the CS 120$^{TM}$ liquid-based cytology system could not pass the technical assessment stage (phase I) of the project. To deal with this, density gradient centrifugation was set up to remove the excess polymorphs, bacilli and background material for the remaining thirty-five CS 120$^{TM}$ cervical samples of the project. The density gradient solution is added to the cervical sample and then the cervical sample is centrifuged at high speed.

The success of the additional step (i.e. density gradient centrifugation) in processing CS 120$^{TM}$ samples is evident in the last thirty-five slides of the project. The staining of CS 120$^{TM}$ slides also improved with the introduction of density gradient centrifugation. Figures 35, 36, 37 and table 12 below show this.

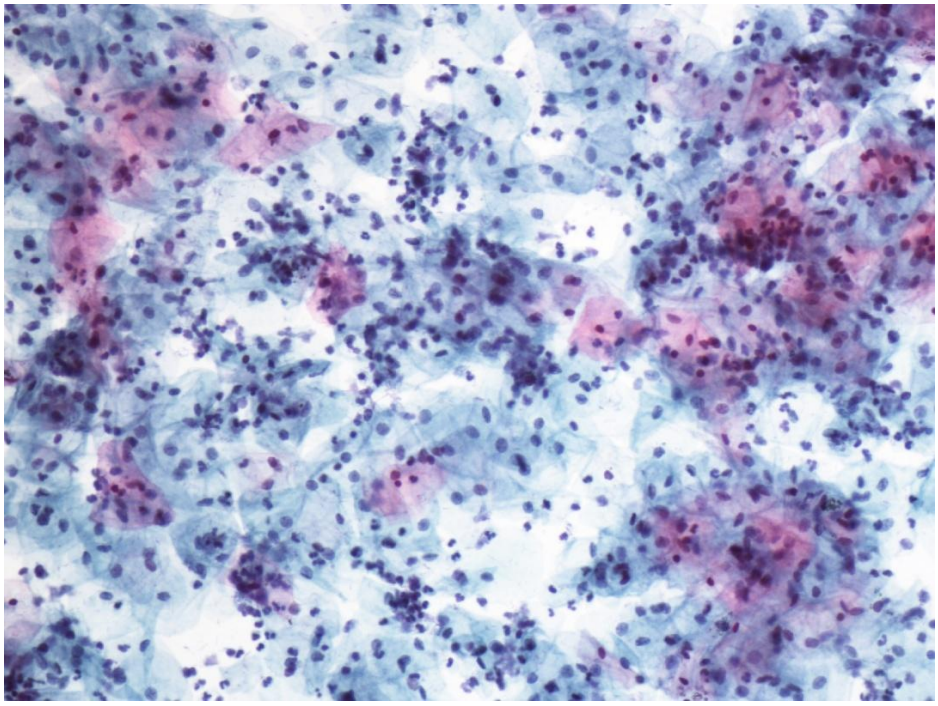


Figure 35: Squamous cells obscured with neutrophils on CS 120™ sample (20x) without using density gradient centrifugation

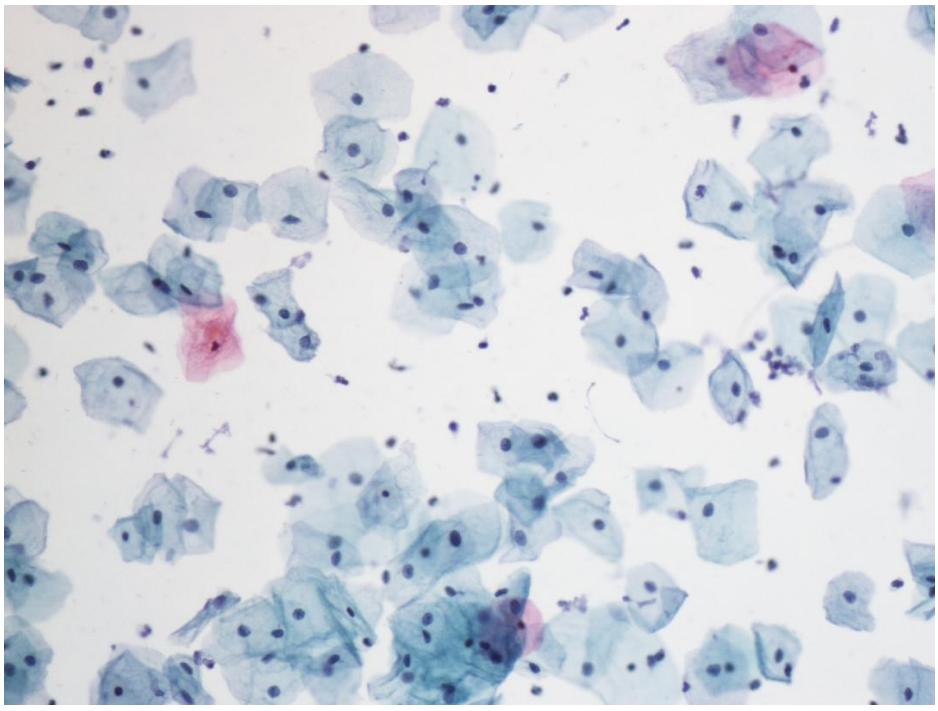


Figure 36: Polymorphs and debris are removed with density gradient centrifugation and unobscured squamous cells are seen in the above CS 120™ slide (20x)

Table 12: Comparison of CellSolution 120™ (CS 120) samples treated with and without density gradient centrifugation (DGC) with corresponding SurePath™ (SP) / ThinPrep™ (TP) samples

| No. | Sample No. | CS/SP/TP | 1st/2nd sample | Diagnosis | Cell preservation | Cytoplasm staining Blue/green | Cytoplasm staining Pink/Orange | Nuclear staining | Background material | Obscuring |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 79 | CS | 1st | BNC-Mild | ok | Ok -pale | Ok- pale | Good | Polys++, bacilli | + |
| | 79 | CS with DGC | | Negative | Holes+ otherwise fine | Good | Good | Good | Polys+, bacilii | + |
| | 12 | SP<br><br>(1 blue semi-o-?bnc or multinucleated ec on top of squamous cells) | 2nd | Negative, Colp-NAD | good | Good | Good | Good | Debris, RBCs,infl cells+ | Occasional |
| B | 80 | CS | 1st | Negative | Good | Good | Good | Good | +- Debris | - |
| | 80 | CS with DGC | | Negative | Very good | Very good | Very good | Very good | - | - |
| | 13 | TP | 2nd | Negative | Good | Ok | Very good | Good | - | - |
| C | 81 | CS | 1st | BNC, koilocytes, ems | Good | Good | Good | Good | +- polys | |
| | 81 | CS with DGC | | BNC, koilocytes, ems | Very good | Very good | Very good | Very good | +- polys | occasional |
| | 14 | TP | 2nd | Mild dyskaryosis. The follow-up smear was negative and bx was not taken | Good | Good | Good (occ hue) | Good | +- poys, debris | A few cells |

Table 12: Comparison of CellSolution 120™ (CS 120) samples treated with and without density gradient centrifugation (DGC) with corresponding SurePath™ (SP) / ThinPrep™ (TP) samples (continued)

| No. | Sample No. | CS/SP/TP | 1st/2nd sample | Diagnosis | Cell preservation | Cytoplasm staining Blue/green | Cytoplasm staining Pink/Orange | Nuclear staining | Background material | Obscuring |
|---|---|---|---|---|---|---|---|---|---|---|
| D | 82 | CS | 1st | Negative | Good | Good | Good | Good | +- polys | occasional |
| | 82 | CS with DGC | | Negative | Very good | Very good | Very good | Very good | occasionally | - |
| | 15 | TP | 2nd | Negative | Good | Overall lighter than the corresponding CS 120 sample | | | +- polys | occasional |
| E | 83 | CS | 2nd | BNC, koilocytes Punch bx- wart virus changes | good | Good | Good | Good | ++- bacilli, polys | Few |
| | 83 | CS with DGC | | BNC | Very good | Very good | Very good | Very good | Polys and debris occ | _ |
| | 16 | SP (infl/dege changes, | 1st | Negative | Very good | Very good | Very good | Very good | Polys+ | - |
| F | 84 | CS | 2nd | Negative The follow-up smear was negative and bx was not taken | Ok | Good | Good | Good | ++- polys, +- debris | + |
| | 84 | CS with DGC | | Negative | Good, halo | Very good | Very good | Very good | +- polys | occasional |
| | 17 | TP | 1st | Borderline | Very good | Very good | Lighter | Very good | occasionally | - |
| G | 85 | CS | 1st | Negative | OK | Good | Good | Good | ++- polys | + |
| | 85 | CS with DGC | | Negative | Very good | Very good | Very good | Very good | few | - |
| | 18 | SP | 2nd | Negative | Very good | Very good | Good | Very good | - | - |

Table 12: Comparison of CellSolution 120™ (CS 120) samples treated with and without density gradient centrifugation (DGC) with corresponding SurePath™ (SP) / ThinPrep™ (TP) samples (continued)

| No. | Sample No. | CS/SP/TP | 1st/2nd sample | Diagnosis | Cell preservation | Cytoplasm staining Blue/green | Cytoplasm staining Pink/Orange | Nuclear staining | Background material | Obscuring |
|---|---|---|---|---|---|---|---|---|---|---|
| H | 86 | CS | 1st | BNC-Mild, koilocytes, candida | Good | Good | Good | Good | +- polys | + |
| | 86 | CS with DGC | | BNC-Mild, koilocytes | Good | Very good | Very good | Very good | ++- polys | ++ |
| | 19 | TP | 2nd | Mild dysk. The follow-up smear was BNC. Bx was not taken | Very good | Very good | Very good | Very good | + polys | + |
| I | 87 | CS | 1st | Inadequate | Very poor | poor | Good | Good | +++- polys, debris | ++ |
| | 87 | CS with DGC | | Negative | Good | Good | Good | Good | ++- polys, debris | + |
| | 20 | TP | 2nd | Negative | Ok | Good | Ok | Good | ++- polys, RBC, debris | + |
| J | 88 | CS | 1st | Negative | Good | Very good | Good | Good | Occ debris | - |
| | 88 | CS with DGC | | Negative | Very good | Very good | Very good | Very good | - | - |
| | 21 | TP | 2nd | Negative | Good | Overall faint staining | | | - | - |

The number of inadequate CellSolution 120™ samples was reduced after the introduction of density gradient centrifugation. The inadequacy rate for 100 CellSolution 120™ slides was 20%, where density gradient centrifugation was not used. However, the inadequacy rate was 5.71% after the introduction of density gradient centrifugation (n= 35). This is shown below in figure 37.

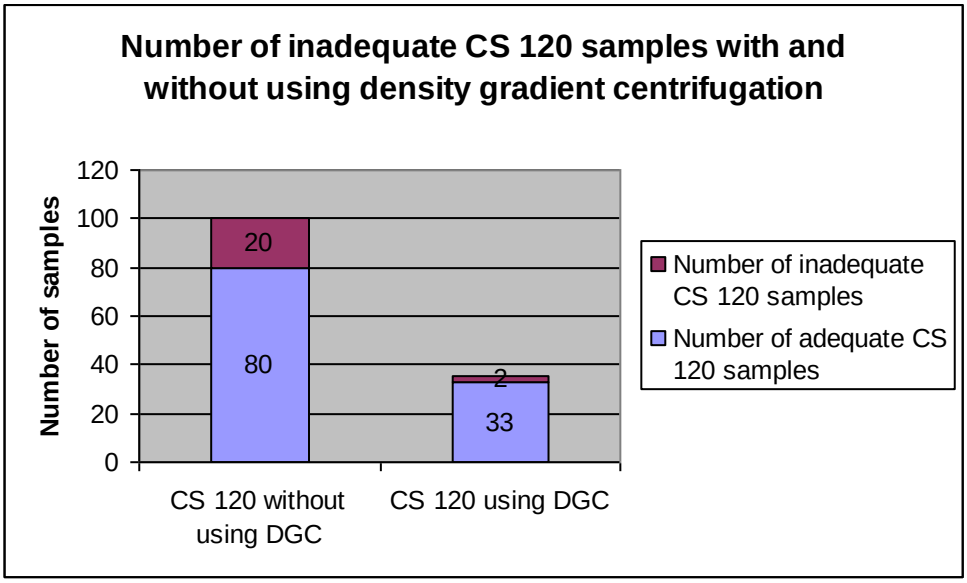



Figure 37: Comparison of inadequate CellSolution 120™ samples with and without using density gradient centrifugation

4. **Labels peel off from the preserved primary tubes:**

The labels on the preserved primary tubes did not stick properly. This is shown in figure 38 below.

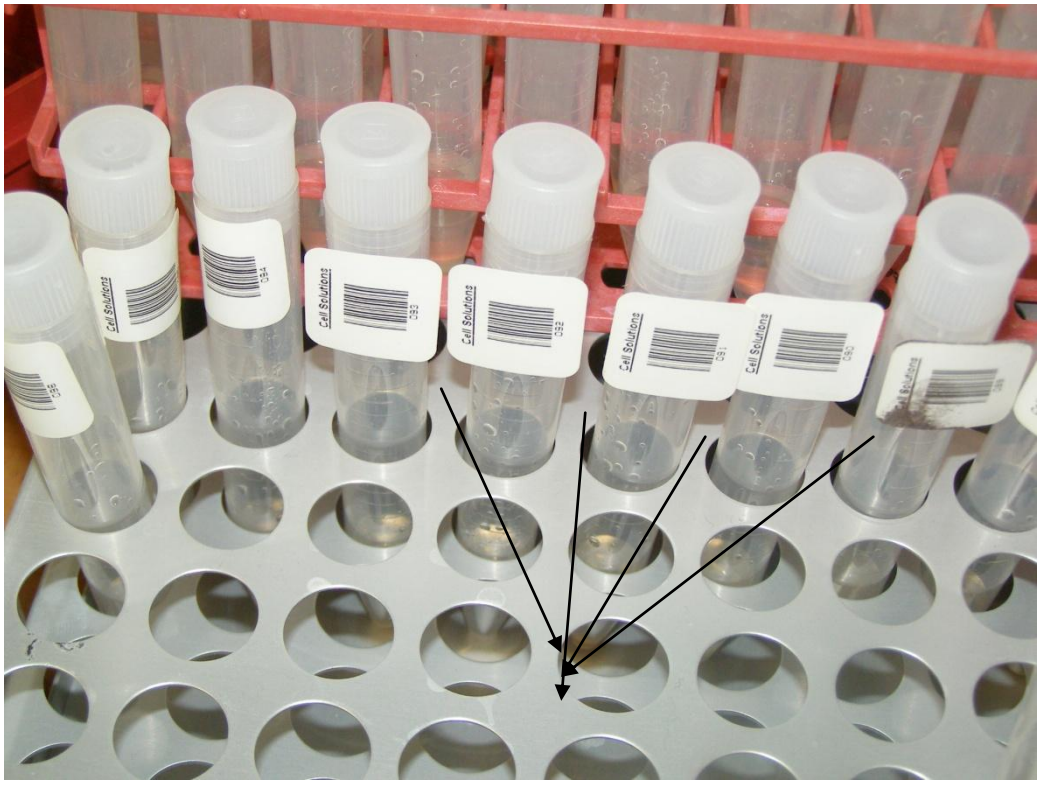



Figure 38: The primary tubes containing the preserved samples showing the peeling off the labels

The primary tubes will need to have a different coating or better glue/adhesive for the labels so that they do not peel off.

The results are discussed in the following chapter.

# DISCUSSION

## 4.1 Background information

Liquid-based cytology (LBC) has been a wonderful aid in cytology. The liquid-based cytology recommended by the National Institute for Clinical Excellence (NICE) in the UK reduced the inadequate cervical rate in England to 2.5% in 2008-2009 (12). Prior to the introduction of LBC, inadequate rates were between 9% and 10%. The NICE recommended the use of two liquid-based cytology systems, i.e. SurePath™ and ThinPrep™ because the inadequate rate reduced with LBC, which reduced the cost overall in National Health Service Cervical Screening Programme. However, the NICE did not recommend any one LBC system over the others. The competition for the liquid-based cervical cytology systems in the UK is limited to SurePath™ and ThinPrep™ LBC, which has led to a high price for each liquid-based cytology sample. Therefore, this project was started to evaluate CellSolution 120™, a new liquid-based cytology system at Manchester Cytology Centre. The project will determine whether CS 120™ is at least equally or more efficient than the existing liquid-based cytology systems (SurePath™ and ThinPrep™) in terms of technical and cost effectiveness (pre-phase I and phase I). If successful in pre-phase I and phase I, the new liquid-based cytology system could be clinically evaluated (phase II). The new liquid-based cytology system could raise competition and reduce the cost per cervical sample in the current economic climate if it is successful in clinical evaluation (phase II).

This project was designed to provide robust and unbiased results in determining the technical effectiveness of the new liquid-based

cytology system, CellSolution 120$^{TM}$. The method was divided in two parts: firstly, the technical requirements (pre-phase I) of the CS 120$^{TM}$ were reviewed (instrument specifications and sample processing); secondly, the technical assessment (phase I) of the CS 120$^{TM}$ machine (macroscopic and microscopic quality) was carried out. One hundred electronically randomised cervical samples were collected from two colposcopy clinics and the nurse smear clinic. The number of samples used in this project was sufficient to validate the technical qualities of the CellSolution 120$^{TM}$ machine. Split samples were not used for the study. Instead, two cervical samples in random order were collected from each woman to avoid any bias in this project. The results showed that the sample preparation on the CellSolution 120$^{TM}$ machine is better with integrated density gradient centrifugation. Integrated density gradient centrifugation was not originally described in the CellSolution 120$^{TM}$ manual.

## 4.2 Primary Outcome

This study shows that the CS 120™ system is easy to operate. This device produces 120 unstained slides in one run, which usually lasts for about two and half hours after the initial preparation. The whole process takes around three and half hours if carried out by a single individual.

The cervical sample is collected in an ethanol based preservative vial. The preservative vial can be stored for two years prior to use, and for thirty days once a sample has been inserted. The collecting device, preservative vials and the end results for comparison between conventional smear, SurePath™, ThinPrep™ and CellSolution 120™ liquid-based cytology systems are shown in figures 39 and 40 below.

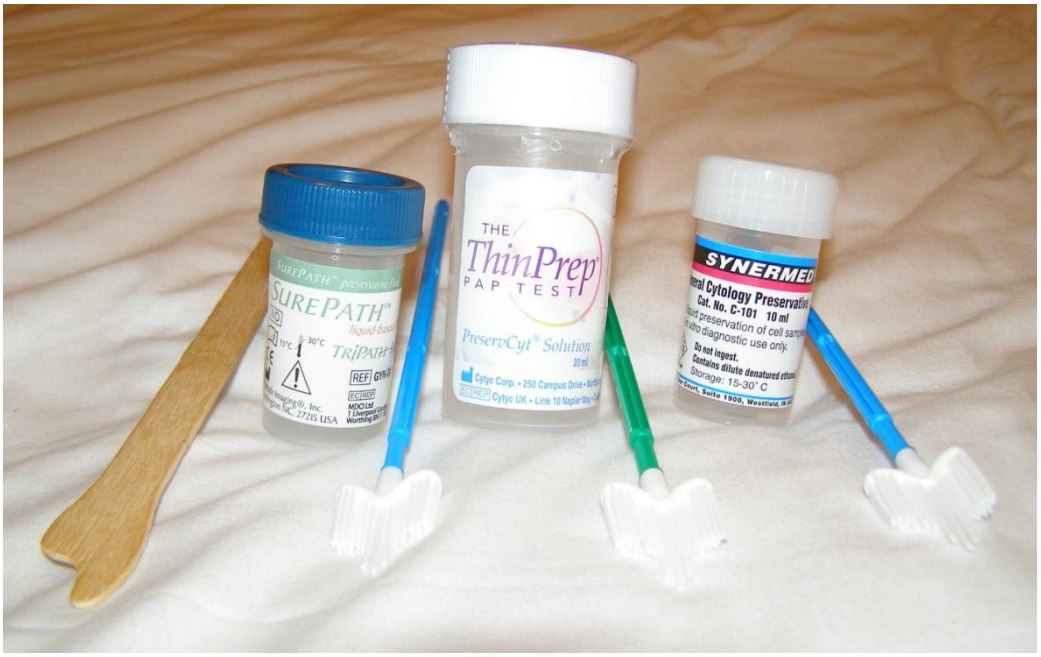



Figure 39: The collecting device and preservative vials for conventional smear, SurePath™, ThinPrep™ and CellSolution 120™ LBC systems

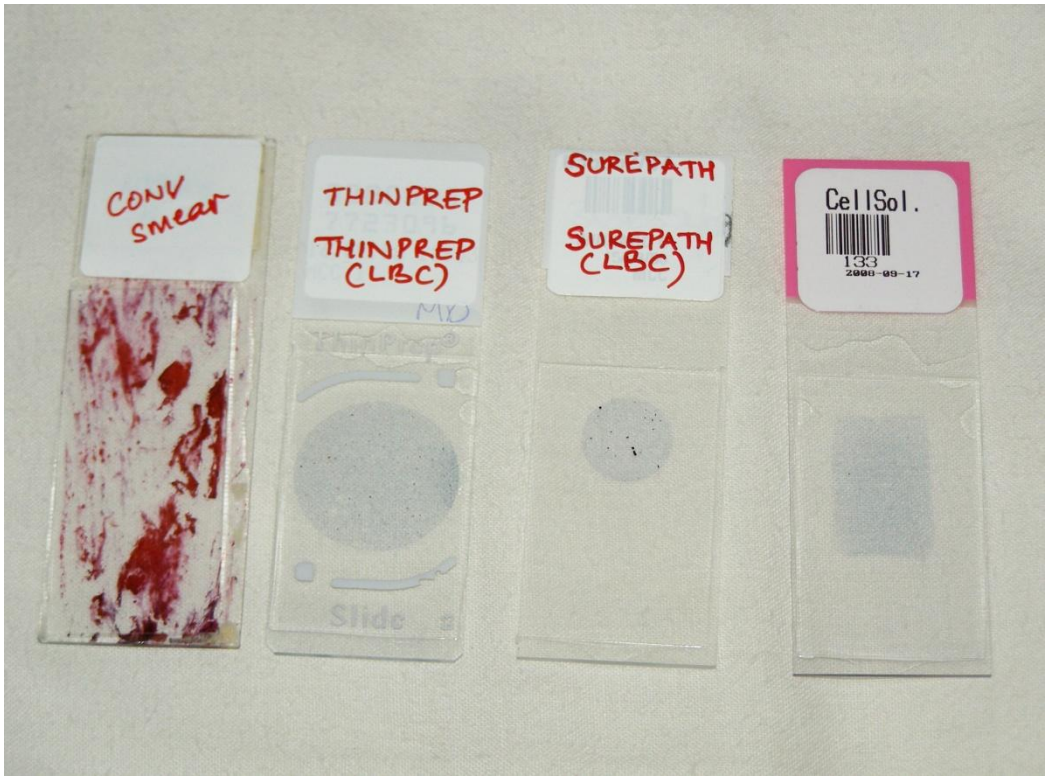



Figure 40: A conventional stained smear and stained samples prepared with ThinPrep™, SurePath™ and CellSolution 120™ LBC systems

A comparison between the three liquid-based cytology systems, SurePath™, ThinPrep™ and CellSolution 120™ is shown in table 13 below.

Table 13: Comparison between SurePath™, ThinPrep™ and CellSolution 120™ liquid-based cytology systems

| **ThinPrep™** | **SurePath™** | **CellSolution 120™** |
|---|---|---|
| Methanol as a preservative | Ethanol as a preservative | Ethanol as a preservative |
| Cervex-Brush® head is rinsed in the vial | Cervex-Brush® head is left in the vial | Cervex-Brush® head is left in the vial |
| 1.9 cm diameter circle of cells | 1.3 cm diameter circle of cells | 12x20 mm rectangular smear |
| Positively charged slides are used | Pre-coated slides are used | No pre-coated or positively charged slides are used |
| T2000- barcode is manually pasted on slide<br>T3000- barcode is automatically fixed on slide | Barcode labels are manually pasted on the slides | Barcode labels are automatically fixed |

Table 13: Comparison between SurePath™, ThinPrep™ and CellSolution 120™ liquid-based cytology systems (continued)

| **ThinPrep™** | **SurePath™** | **CellSolution 120™** |
|---|---|---|
| Individual laboratory stain can be used, slides not stained by the machine | Company stain has to be used, slides are stained by the machine | Individual laboratory stain can be used, slides not stained by the machine |
| Any cover slip-24x40 mm to be used | Company provided cover slip 22x50mm to be used | Any cover slip can be used |
| Waste to be collected in Genta containers | Waste goes in regular sinks or incinerated | Waste goes in regular sinks or incinerated |
| Residual samples can be stored for a shorter period (4 weeks) | Residual samples can be stored for a longer period (6 months) | Residual samples can be stored for a shorter period (4 weeks) |
| Only vials to be stored | Vials and test tubes to be stored | Only test tubes to be stored |
| Less space required | More space required | Medium space required |
| Cell filtration method is used | Cell enrichment process is used | Dilution and density gradient centrifugation is used |

Table 13: Comparison between SurePath™, ThinPrep™ and CellSolution 120™ liquid-based cytology systems (continued)

| **ThinPrep™** | **SurePath™** | **CellSolution 120™** |
|---|---|---|
| T2000- labour intensive<br>T3000- labour free | Labour intensive process | Moderately labour intensive, but, with density gradient centrifugation, it is labour intense process |
| Well-demarcated edge- no drift | Drifting of cells seen | Drifting of cells is occasionally seen |
| Holes between cells | No holes between cells | Holes between cells are seen in some cases |
| Less 3 dimensional effect seen | More 3 dimensional effect seen | Less 3 dimensional effect seen |
| Less need to use high-power | Need to use high-power more often | Less need to use high-power |
| Metaplastic cells – a difficult area | Hyper chromatic crowded groups- a difficult area | A difficult area is not recognised yet |

Table 13: Comparison between SurePath™, ThinPrep™ and CellSolution 120™ liquid-based cytology systems (continued)

| **ThinPrep™** | **SurePath™** | **CellSolution 120™** |
|---|---|---|
| Maximum capacity per year<br>T2000- 30,000 samples<br>T3000- 60,000 samples | Maximum capacity per year<br>72,000 samples | Maximum capacity per year- 62,400 samples if two runs/day and 90,000 if three runs/day |

The CS 120™ system is similar to SurePath™ in terms of collecting the cervical sample and preservation. Also, it is similar to SurePath™ in preparation of the sample (density gradient centrifugation). However, the CS 120™ slides are to be stained separately, unlike with SurePath™. Microscopically, the preparation and cells in CS 120™ look similar to those seen in ThinPrep™. Therefore, it is certain that if the CS 120™ preparations are cost and clinically effective, accepting CS 120™ as a newer liquid-based cytology system in NHSCSP will be easy.

The maintenance of the CS 120™ device, data recording and retrieval are user friendly. The cost of the machine is unknown, however the cost of the reagents appears similar to SurePath™. The reagents used in the CS 120™ system are tap water, glucyte and density gradient solution. All the reagents are stored at 15-30 degrees celsius. Glucyte is a unique, non-toxic mixture of polymers, which is designed to suspend the cells in an isotonicaly balanced self-adhering matrix that affixes itself

to glass slides. It is permeable to traditional stain but does not retain the stain itself. However, the study shows that glucyte retains some stain and if the glucyte tubes are not cleaned with tap water at the end of the run, the pumping tubes are blocked.

The current study found that 4.95 cells are enough per 40 high power field at 22x objective to label a CS 120$^{TM}$ slide as adequate. While, 4.18 and 8.95 cells are required to label a ThinPrep$^{TM}$ and SurePath$^{TM}$ sample respectively as adequate. The calculations are recorded below as per the Bethesda system (39) (where 5000 cells are considered as adequate) in table 14.

Table 14: Guideline for estimating cellularity of CellSolution 120$^{TM}$ sample (39)

1)-number of cells required per field= 5000/area of circle/area of ocular

2)-diameter of an ocular or microscopic field in mm is the field number of the eyepiece/magnification of the objective

3)-area of the field= area of circle (pi x radius$^2$)

| **Prep diam** | **Area** | **Fn20 eyepiece/10x objective** | | **Fn20 eyepiece/40x objective** | | **Fn22 eyepiece/10x objective** | | **Fn22 eyepiece/40x objective** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Fields@ Fn20 10X** | **Cells/fields for 5000** | **Fields@ Fn20 40X** | **Cells/fields for 5000** | **Fields@ fn22 10X** | **Cells/fields for 5000** | **Fields@fn22 10X** | **Cells/fields for 5000** |
| | | | | | | | | | |
| 13 (SP) | 132.665 | 42.25 | 118.34 | 676.00 | 7.40 | 34.92 | 143.2 | 558.68 | 8.95 |
| 19 (TP) | 283.385 | 90.25 | 55.40 | 1424.05 | 3.51 | 74.59 | 67.03 | 1195.71 | 4.18 |
| | | | | | | | | | |
| **CELLSOLUTION 120** | | | | | | | | | |
| | | | | | | | | | |
| 15X20 (as stated) | 300 | 95.54 | 52.33 | 1528.66 | 3.27 | 78.96 | 63.32 | 1263.69 | 3.96 |
| 12x20 (normally) | 240 | 76.43 | 65.42 | 1222.93 | 4.09 | 63.17 | 79.15 | 1010.95 | 4.95 |
| | | | | | | | | | |

Pi value= 3.14, FN= field number

### 4.3 Discordant results and the reasons contributing to them

- **Printer-**

The robotic arm was unable to pick up some labels and paste them on the slides. This was initially encountered, as there was no support for the label reel, and the labels slipped off the printer head. Putting hinges onto the printer head to support the label reel solved the label uptake failure.

- **Macroscopic findings-**

Holes or gaps were found on some smears. They were thought to be due to:

- Air bubbles in the glucyte line, which may result in the glucyte not being properly added to the sample. This results in incomplete fixation of the sample on the slide.
- Incomplete drying prior to staining
- Over vigorous washing during staining
- Thick smears perhaps not taking sufficient glucyte

It is possible to hypothesise that if such a gap is created on the smear while staining, it may result in removal of abnormal cells from that area. The removed area may stick to another slide and give an impression of abnormality in a normal smear. However, this may occur only infrequently as it would require some agent to enable the removed area of smear to stick to another slide. Nevertheless, the removal of abnormal cells can occur with the formation of a gap while staining.

- **Microscopic findings-**

Variable staining was observed in a few of the stained CS 120$^{TM}$ slides. Excess polymorphs and bacilli were seen in many CS 120$^{TM}$ slides and this hindered staining. The reason for this was not clear but it may have something to do with the interaction between polymorphs, bacilli and the dyes.

Furthermore, obscuring elements like polymorphs, bacilli and background material were seen on the cervical cells, which made assessment of the CellSolution 120$^{TM}$ slides very difficult. The CellSolution 120$^{TM}$ slides with such appearance were not acceptable and could not pass the technical assessment (phase I) of our evaluation project. The presence of obscuring elements resulted in a high inadequate rate with CellSolution 120$^{TM}$ as compared to the existing SurePath and ThinPrep liquid-based cytology systems. A high rate of inadequacy is usually not expected in the liquid-based cytology system according to the NICE guideline. The reasons contributing to cell obscurement in CS 120$^{TM}$ slides are discussed below-

The CS 120$^{TM}$ device prepares the cervical sample by centrifugation and decanting the supernatant after centrifugation. This preparation step removes a little obscuring debris. The dilution of water and ultrasonic sensor makes adjustments in cellularity, but do not remove any debris. However, there is not enough barrier to remove the obscuring elements in CS 120$^{TM}$ (such as ultra filtration in ThinPrep$^{TM}$ and the cell enrichment process in SurePath$^{TM}$). As a result, the squamous cells were frequently obscured by neutrophils and debris in

the CS 120$^{TM}$ slides. Obscured squamous cells made microscopic interpretation difficult. It was noted that excess polymorphs and bacilli even interfered with staining.

- **Other-**

There are certain important steps during the initial preparation of the cervical sample before it is loaded on CS 120$^{TM}$ machine. These are decanting and vortexing in the CS 120$^{TM}$ liquid-based cytology system. The preparations vary with different individual and also with the same individuals at different times if the preparation steps are not performed properly. Figure 41 below is shown to support the statement.

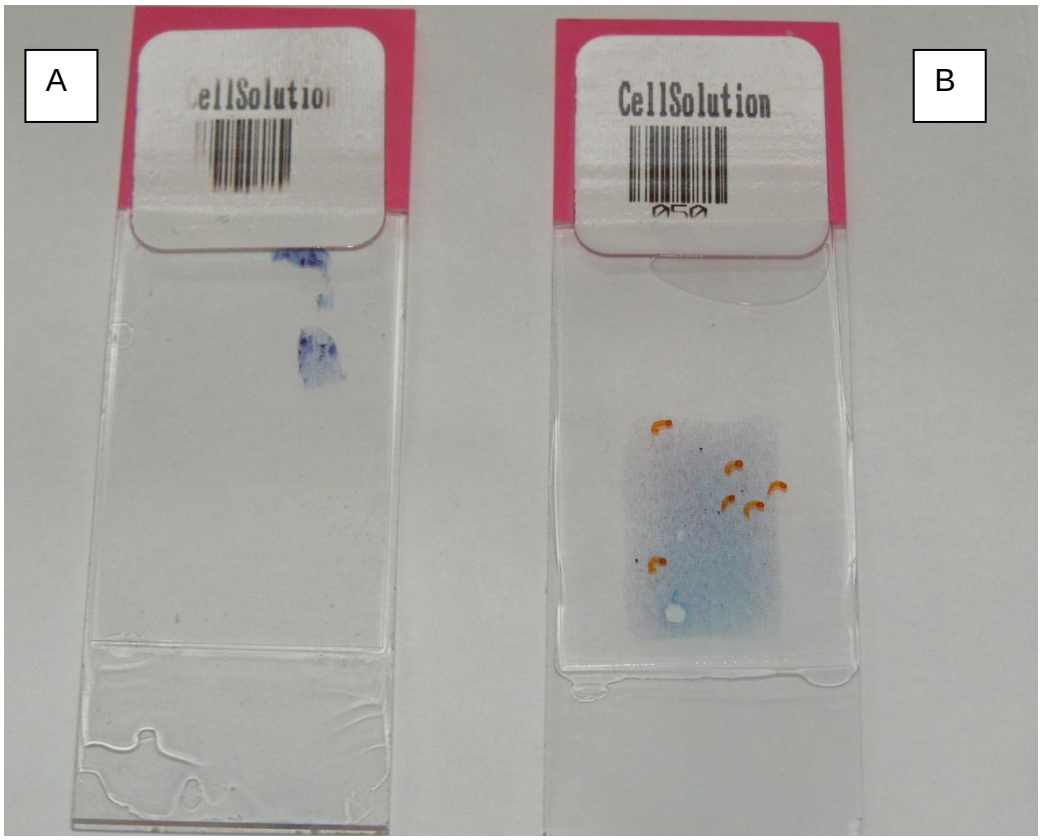



Figure 41: CS 120$^{TM}$ sample and its repeat preparation

The same individual has prepared both the preparations. However, sample (A) on the left is inadequate and its repeat preparation on the right (B) is entirely acceptable even though it has tiny gaps. The obscuring elements are removed during decanting the supernatant after centrifugation and mixing of the cells occurs during vortexing.

The labels do not remain adhered to the preserved primary tubes. This may be attributed either to the inherent quality of the tubes or the labels. The problem needs to be resolved because if the labels peel off the tubes, it will not be possible to re-process any CS 120$^{TM}$ sample when needed either for teaching or diagnostic purposes.

The robotic pipette draws the smear from periphery to centre (observation) and then picks the extra sample from the centre. This preparation makes the peripheral part of the CS 120$^{TM}$ smear thinner than the central part. Therefore, the peripheral part of a CS 120$^{TM}$ slide dries quicker than the central part and due to its early drying, the peripheral part is lighter stained than the crispy and well-stained central part of the smear. This is shown in figure 42a and 42b below.

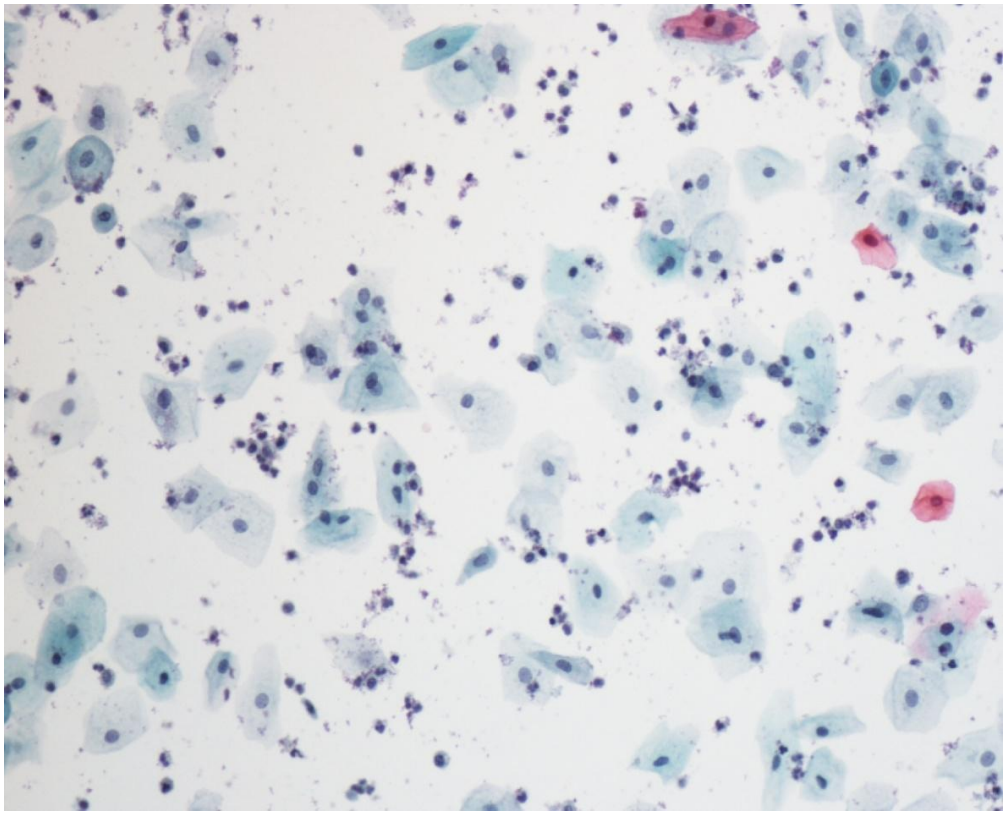


Figure 42a: Peripheral part of the CS 120™ sample (20x)

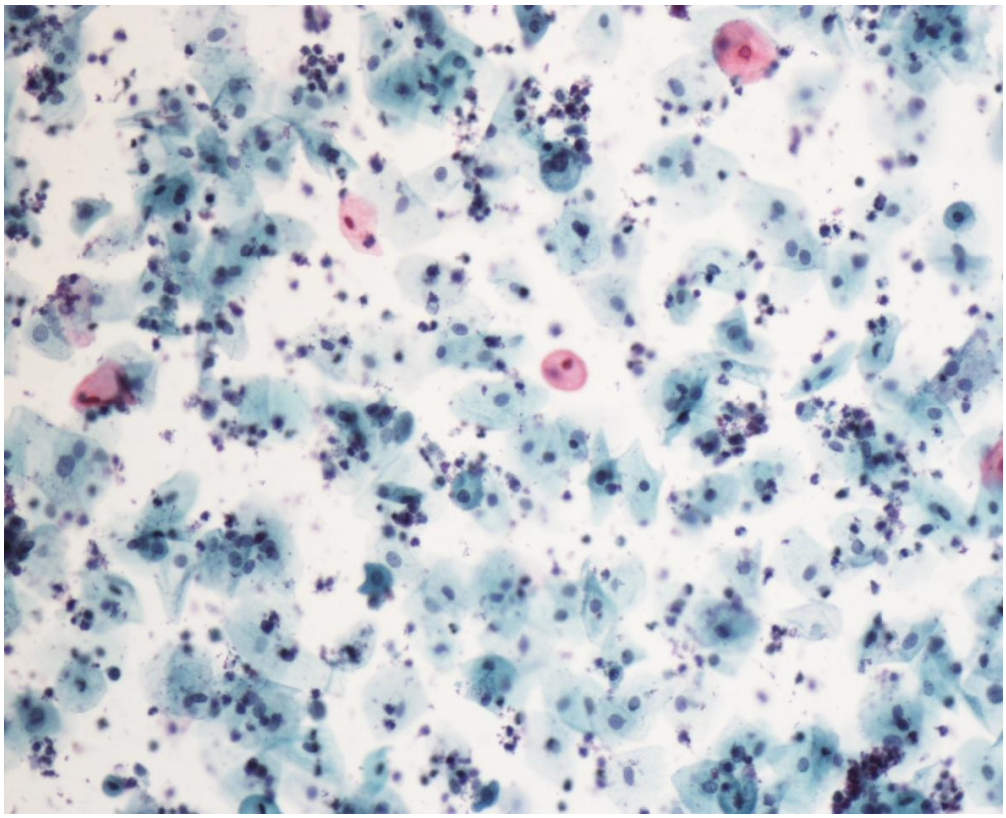


Figure 42b: Central part of the CS 120™ sample (20x)

The CS 120™ liquid-based cytology system takes more time to dry the prepared slides than stated in the CS 120™ user manual.

### 4.4 Solutions for the discordant results

- **Printer-**

The introduction of hinges on the printer head to support the label reel made a difference in robotic uptake of labels. Consequently, label uptake failure was not encountered.

- **Macroscopic findings-**

The addition of more glucyte and a longer drying time solved the problem of small or tiny holes on the CS 120$^{TM}$ slide. The addition of more glucyte was tackled by increasing the number of robotic arm dips into the secondary tubes, which contain glucyte.

- **Microscopic findings-**

Density gradient centrifugation is used in the SurePath$^{TM}$ liquid-based cytology system and so the concept is known to liquid-based cytology users. The same concept of density gradient centrifugation was thought of use with the CellSolution 120$^{TM}$ liquid-based cytology system by the company. However, the company was worried that during the decanting stage of the supernatant, scanty dyskarytotic cells may be removed. Therefore, density gradient centrifugation step for preparation of CS 120$^{TM}$ slides was omitted during the final development of the machine. However, density gradient centrifugation step was re-introduced by us and set up to remove excess polymorphs, bacilli and background material for the last thirty-five CS 120$^{TM}$ cervical samples of

our project. Density gradient solution was added to the cervical sample and then the cervical sample was centrifuged at high speed. This centrifugation allowed the separation of molecules with varying weights. The heavy weight cells (e.g. squamous cells) settled at the bottom of the tubes and light weight cells (e.g. polymorphs, red blood cells, debris) remained at the top as the supernatant. This supernatant was then aspirated with a pump aspirator.

**Method of using density gradient centrifugation in the CellSolution 120$^{TM}$ liquid-based cytology system:**

Density gradient solution was added to the cervical sample and centrifuged at 1100 revolutions per minute for 2 minutes. As a result, elements of low weight like polymorphs, red blood cells and bacilli were trapped above the heavy weight squamous cells. This supernatant of trapped debris was aspirated with an aspirator. Later on, the sample was centrifuged (at 2150 rpm for 10 minutes) and decanted to obtain a cell pellet without obscuring elements. Then the sample was vortexed so that it could be loaded on the CS 120$^{TM}$ machine after initial homing. The success of the additional step (i.e. density gradient centrifugation) in processing was evident in the last thirty-five slides of the project.

**Advantages of using density gradient centrifugation in the CellSolution 120$^{TM}$ liquid-based cytology system:**

- Polymorphs and debris are removed
- The number of inadequate samples is reduced

- Overall cellularity remains the same
- Staining improves as polymorphs and debris are removed
- The dyskaryotic cells are easily recognised
- The three dimensional effect is reduced

The density gradient centrifugation system on the CS 120$^{TM}$ LBC system has also helped in good staining of the sample as shown in figure 43 below.

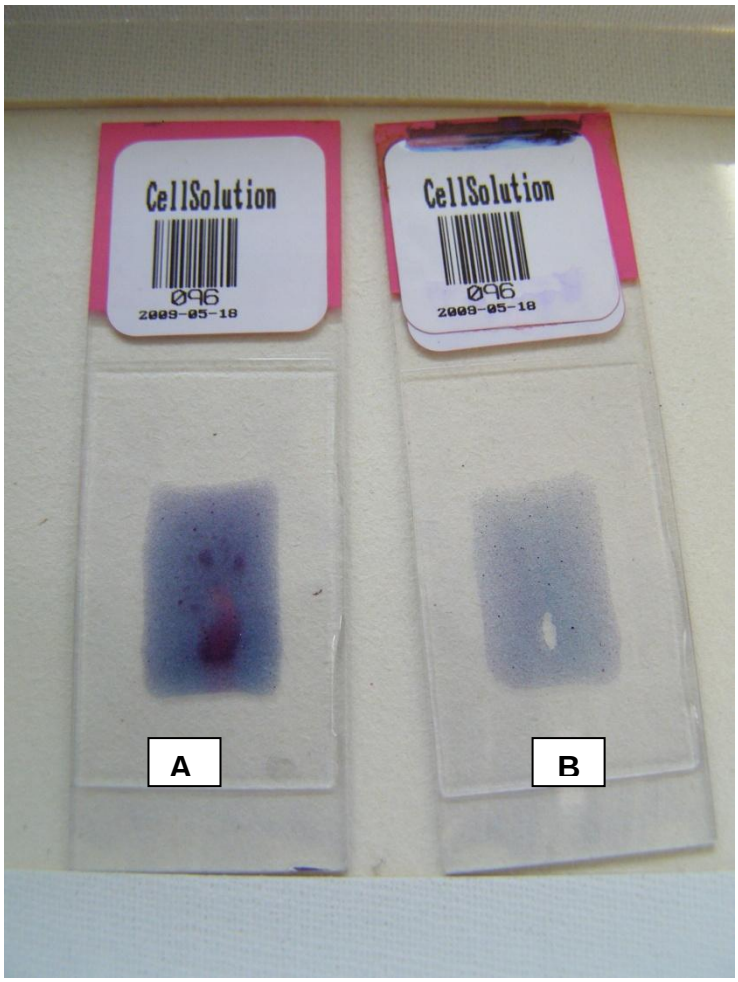



Figure 43: A- CS 120$^{TM}$ sample prepared without density gradient centrifugation, showing variable staining, B- CS 120$^{TM}$ sample prepared with density gradient centrifugation showing even staining intensity

It is thought that excess polymorphs, bacilli and background material make the smear thick, and this does not allow the stains to penetrate sufficiently. But, when the debris and background materials are removed with density gradient centrifugation, the smear becomes thin and allows an appropriate and consistent amount of stain to penetrate.

**Drawback of using density gradient centrifugation in CellSolution 120$^{TM}$ system:**

- The process of producing slides is labour intensive and more time consuming.

The advantages of using integrated density gradient centrifugation in the CellSolution 120$^{TM}$ liquid-based cytology system outweigh the drawback. Therefore, it is thus suggested that the CS 120$^{TM}$ device should integrate density gradient centrifugation as a routine process in preparing the cervical slides.

- **Other-**

Decanting and vortexing are important steps of sample preparation in the CellSolution 120$^{TM}$ liquid-based cytology system. If they are not performed properly, the CS 120$^{TM}$ sample will have many obscuring elements and these will make it difficult for the sample to stick on the slide with glucyte.

### 4.5 Unexpected outcome

The unanticipated finding in our study was the difficulty in obtaining the cervical samples for this trial. Moreover, the CS 120$^{TM}$ device was not producing acceptable slides initially. However, following the introduction of support to the printer head and density gradient centrifugation, CS 120$^{TM}$ machine started preparing slides of an acceptable standard.

With the small sample size in phase 1 of our project, caution must be taken, as the findings may or may not be transferable to the samples in clinical assessment (phase 2).

## 4.6 Comparison with the other study on the CellSolution 120™ liquid-based cytology system

There is a recent study on Glucyte™ by Joel et al (40), in which they evaluated and compared the Glucyte™ method to both SurePath™ and ThinPrep™. Joel et al used a split sample with a manual glucyte method. The authors concentrated the unused ThinPrep and SurePath samples by centrifugation, decanted them and then mixed and diluted them with glucyte. Later on, they applied the mixture to the slide and allowed it to dry into a 16-18 mm circle. The slides were stained with modified Pap stain. Their study had 303 samples to show the efficiency of Glucyte™. Thirty samples which were evaluated cytologically were compared using the Digene Hybrid Capture II™ high risk HPV assay. The authors concluded that the method is practical, inexpensive and easy to use. Their study showed equivalent sensitivity and specificity for the detection of squamous intraepithelial lesions when compared to both of the current liquid-based cytology systems. With these results, they supported the future value and utility of the Synermed GluCyte™ thin-layer liquid-based cytology preparation in gynecologic applications. However, the authors make no statement about the quality of the slides prepared with Glucyte™ in their study. Therefore, it would be necessary to evaluate the system technically before moving on to suggest clinical effectiveness. Also, it is possible that the cytology samples in the study by Joel et al were categorised using the Bethesda classification, where there is a category known as “Adequate sample obscured by polymorphs”. There is no such category in the British Society for Clinical

Cytology. Therefore, our study and its results will form the basis of a continued evaluation of CellSolution 120$^{TM}$ in the clinical effectiveness phase (phase II) in the UK.

# SUMMARY AND CONCLUSION

## 5.1 Summary

The second-generation liquid-based cytology systems must undergo thorough evaluation to be introduced for cervical screening in the UK. Initially, there were four second-generation liquid-based cytology systems for evaluation. However, the manual methods would not serve large population screening and so were not evaluated any further. Therefore, CellSolution 120$^{TM}$ was the only product available in the UK at the time, which had the potential utility to be included in the National Health Services Cervical Screening Programme (NHSCSP). Full ethics approval was granted for the evaluation of second-generation liquid-based cytology systems. Central Manchester and Manchester Children's University Hospitals was the sponsor of the project. This study was funded by the NHS Purchasing and Supply Agency, Centre for Evidence based Purchasing on behalf of NHSCSP. The project was managed by Guildford Medical Device Evaluation Centre.

The project started with the evaluation of the CellSolution 120$^{TM}$ liquid-based cytology system in terms of technical and clinical effectiveness. The technical evaluation of CellSolution 120$^{TM}$ was divided into pre-phase I and phase I and these phases were dealt within this thesis. The colposcopy and nurse smear clinic cervical samples were included in the technical evaluation. The pre-phase I requirements as set by the NHSCSP were satisfied by the CellSolution 120$^{TM}$ liquid-based cytology system. Therefore, further evaluation (phase I) in terms of reliability, quality of preparation and reproducibility of one hundred CellSolution 120$^{TM}$ slides was carried out. During this evaluation, the

CellSolution 120™ slides initially showed polymorphs and debris resulting in inadequate samples. However, the CellSolution 120™ slides demonstrated that cell presentation was comparable to currently used LBC systems in the UK once a density gradient cleaning procedure was included at the sample preparation stage. This density gradient centrifugation was not listed in the CellSolution 120™ user manual. The density gradient centrifugation was tested on thirty-five CellSolution 120™ samples and was shown to remove background debris and resulted in cleaner and more effectively stained slides. .

## 5.2 Conclusion

The protocol for evaluation of second-generation liquid-based cytology systems has been well laid out by the National Health Service Cervical Screening Programme. The protocol was divided for thorough evaluation of the second-generation liquid-based cytology system for technical and clinical components. The method of evaluation for the second-generation liquid-based cytology systems for the technical evaluation was divided into pre-phase I and phase I.

The majority of the world literature on this subject has carried out the direct comparison of the new liquid-based cytology system with the existing liquid-based cytology systems, conventional cytology or histology. In our opinion, this will not serve any purpose. It will simply suggest whether the new liquid-based cytology system is as clinically effective as the existing liquid-based cytology systems or not. It will fail to address the practical technical issues related to the new system. The protocol adopted in this project evaluated the new-liquid based cytology system in different phases: technical requirements (pre-phase I), technical acceptability (phase I) and clinical effectiveness (phase II). With respect to technical requirements (pre-phase I), sample processing, the usefulness of its user manual, the training and support provided by the company and the cost of the machine were evaluated first. Only if these technical requirements (pre-phase I) laid by the NHSCSP were satisfied by the new liquid-based cytology system will further evaluation be carried out. Phase I of the protocol determines the technical parameters of the new liquid-based cytology system: whether the new liquid-based cytology system is reliable in terms of processing the cervical samples, whether it

is user friendly and whether it is able to reproduce the samples. Therefore, by adopting this protocol, there are many technical issues, which can be known and sorted out. The further evaluation of clinical effectiveness (phase II) should be carried out further only if the pre-phase I and phase I are satisfied by the new liquid-based cytology system to determine the clinical effectiveness of the new liquid-based cytology system in comparison to histological outcome. Overall, the adopted protocol evaluates the new liquid-based cytology system in detail for the practical introduction of the new system in the NHSCSP.

It was through the thorough evaluation of CellSolution 120$^{TM}$, that a new step in processing the CellSolution 120$^{TM}$ slides was found to be better than was originally described in the CellSolution 120$^{TM}$ user manual. It was concluded that the CellSolution 120™ liquid-based cytology system is technically competent to progress to the clinical assessment stage (phase II) with the introduction of density gradient centrifugation into the sample preparation procedure.

## 5.3 Research recommendations

A clinical assessment (phase II) of CellSolution 120$^{TM}$ should be undertaken to show the clinical effectiveness of this new liquid-based cytology system by direct to vial method. The clinical assessment phase should compare the cytology results with the histology results. Funding should be sought to progress to the next stage of clinical assessment.

## 5.4 Implications for the National Health Service Cervical Screening Programme (NHSCSP)

The reagents, namely water, glucyte and density gradient solution are used in preparing the gynaecological slides on the CellSolution 120$^{TM}$ liquid-based cytology system. Tap water can be used. Glucyte and the density gradient solution are easily available and inexpensive. The actual cost of the machine is not known to the researchers. A large number of CS 120$^{TM}$ samples (120 samples) can be processed in one batch. Moreover, human papilloma virus and molecular tests can be performed with CS 120$^{TM}$ sample, which can complement the cytology diagnosis of CS 120$^{TM}$ sample and also help in follow-up after treatment or in borderline nuclear changes. Considering all the above factors along with the acceptable quality of CS 120$^{TM}$ slides, the CellSolution 120$^{TM}$ should be further clinically evaluated. If the results of the clinical evaluation are acceptable, CellSolution 120$^{TM}$ can provide a cheap alternative in cervical sample processing in the current economic climate.

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# APPENDICES

## Appendix A

### NHSCSP technical requirements for LBC systems for cervical screening [1]

## 1 CE marking

All equipment and consumables, including sampling devices, must be CE marked with regard to the IVD Directive [2] where appropriate. Any electrical device which is not an invitro diagnostic device must be CE marked for electrical safety [3].

## 2 Sample collection

### 2.1 Sample collection vials

The supplier must provide:

- collection vials that are in regular use throughout the NHS, or which can be reliably and regularly supplied to the required quantity and quality. It must be obvious when the vial lid is closed.
- vials prefilled with collection fluid and checked for fluid loss and contamination
- vial handling trays to minimise the risk of spillage. A visual recognition system or colour coding system is desirable.
- vial storage requirements, including any restrictions on the number of vials that can be stored together and any limitations.
- control of Substances Hazardous to Health (COSHH) Regulations 2002 [4] data to be available to both laboratories and sample takers
- requirements and/or restrictions for the transportation of vials, both before and after the addition of the sample, including the specification of suitable transport boxes
- advice on disposal of vials and other consumables
- vials having a shelf life of at least 18 months from date of manufacture.

### 2.2 Sampling devices

- The system must use either a broom, an extended tip spatula or a spatula/brush combination. The supplier must state the recommended device for the system and whether or not alternative devices can be used, and must provide reasons for this decision.
- Sampling devices must be those in regular use throughout the NHS, or which can be supplied reliably to the required quantity.
- The sampling device(s) must be capable of transferring an adequate number of cells for screening from the transformation zone of the cervix to the collection vial.

## 2.3 Sampling technique

The supplier must:

- provide detailed instructions on sampling technique and transfer of cells from the sampling device to the collection fluid
- provide training for sample takers
- highlight any differences in methodology between recommended sampling devices.

## 3 System specifications

The supplier must provide the following requirements:

## 3.1 Physical

- Serial number (displayed on instrument)
- Model number (displayed on instrument)
- Voltage, current and fusing requirements (compliant with British Standards)
- Size and space required

## 3.2 Utility and environmental

- floor type and loading
- drainage
- water supply
- electrical supply
- an uninterrupted power supply and electrical filter if required
- waste disposal
- a system capable of operating between 15–35°C and under typical laboratory humidity

## 4 Installation and commissioning

The supplier must:

- install the equipment using their own service engineers or appointed agent
- provide advice and support if the instrument has to be moved after installation
- state the assistance and support to be provided during the commissioning phase, acceptance testing and validation of performance
- satisfactorily demonstrate that the equipment is working within specification before formal handover.

## 5 System operation

The system must be easy to operate and routinely maintained by laboratory staff (biomedical scientists and/or medical laboratory assistants).

## 5.1 Instructions for use (IFU)

The supplier must provide a comprehensive operator manual in English which includes:

- start-up
- calibration
- sample processing
- decontamination
- fault recognition and troubleshooting
- system features that minimise the risk of carry-over between specimens
- waste disposal.

## 5.2 Throughput

- Start-up and shut-down (including decontamination procedures) should not take longer than 15 min per day. Ideally, the system should be capable of running directly from standby.
- Any system must be automated to a degree that comfortably permits the processing of 20 sample vials per hour by a single operator (not including staining).
- The throughput of the system for slide production, staining (if included) and cover slipping (if included) should be provided.
- The supplier must make it clear which processes are included in their system.

## 5.3 Maintenance

The in-house maintenance procedures must be documented in the instructions for use, together with an estimate of the time required.

## 5.4 Error notification and troubleshooting

The system should provide messages that identify common errors to operators. Corrective actions must be documented in the instructions for use.

## 5.5 Consumables

- Working reagents must be supplied ready for use or be simple to prepare.
- Reagents should be stable on board the system for at least three days.
- The shelf life of the reagents should be at least three months upon delivery.
- All consumables must be readily available from the suppliers as stock items.
- Glass slides that are compatible with the system must be specified or supplied.

# 6 Manufacturer/supplier services

## 6.1 Training

The supplier must provide full training in English which includes:

- system operation
- maintenance
- quality control
- waste disposal
- Sufficient slides, covering all diagnostic categories used in the UK, must be provided in accordance with the NHSCSP publication Liquid Based Cervical Cytopathology Training log [5].

## 6.2 Engineering and technical support

The supplier must specify or provide:

- the external service requirements and contracts available, together with guaranteed response times and level of support outside normal working hours
- specific exclusions to the contract
- options for back-up/loan systems must be available should long-term down-time occur (two weeks or more)
- an English speaking point of contact
- all service reports and data relating to routine performance, planned and unplanned maintenance, and fault rectification.

# 7 Slide preparation

## 7.1 Specimen preparation

The system process must:

- be suitable for use with all cervical cytology samples, including those which are heavily blood-stained or mucoid
- have the capability to remove a significant number of polymorphs, blood and mucus
- produce slides that are a representative sample of the epithelial cell content of the original sample
- allow additional slides of equivalent content to be produced from the original sample (for training and quality assurance purposes)
- spread cells evenly on the slide for ease of screening
- hold cells in position so that they do not move once the cover slip has been applied
- produce LBC preparations which are similar to each other when taken from women of the same age
- produce inadequate test rates within the 10th to 90th centile of performance in current LBC laboratories in England
- achieve a **high** and **low** grade pick up rate within the 10th to 90th centile of performance in current LBC laboratories in England; this should be demonstrated in both split sample and

direct to vial studies in various asymptomatic populations, similar to that of the UK, and published in peer review journals or provided for peer review by the NHS.

## 7.2 Staining procedure

- The prepared slides must be compatible with NHSCSP approved staining regimes.
- If slide staining forms part of the system, it must produce cell preparations that meet the standards for liquid based cytology [6].
- The slide and associated slide carriage devices should be suitable for use with automated staining and/or automated cover slipping devices if this is not part of the system.

## 7.3 Labelling

- The vials must carry preprinted labels for documenting patient demographics which conform to UK standards. There must be sufficient space to enter patient details and enable indelible marking with ballpoint pens.
- Systems that generate slide labels must ensure that these always match those for the relevant vial.
- If bar code labels are used, the supplier must be able to provide a system that is compatible with UK systems of labelling, or recommend such a system that is available in the UK.

## 7.4 Quality control

- The reject rate for both single and multisample processors must be less than 2%.
- Calibration and quality control (QC) procedures must be stated.
- The system must provide QC and calibration data.
- The QC results should be clearly indicated with appropriate status flags and should be available for long-term storage for accreditation compliance.

# 8 Health and safety

The supplier must:

- confirm compliance of the system with relevant regulations for electrical, mechanical and biological safety
- for all reagents, confirm compliance with relevant regulations regarding shipping, labelling and information on hazardous substances. COSHH product data sheets and risk management information must be provided
- provide a decontamination protocol for the system with recommendations as to when it should be used
- provide recommendations for handling and disposal of ‘high risk’ samples

- comply with all UK and EC safety regulations and any guidance issued by the Medicines and Healthcare products Regulatory Agency (MHRA)
- ensure that the system does not generate dangerous aerosols.

## 8.1 HAZMAT

- Vial preservative solutions must inactivate viruses that model relevant human pathogens such as human immunodeficiency virus type 1 (HIV-1), hepatitis B virus (HBV) and hepatitis C virus (HCV). As it is not possible to culture HBV and HCV in vitro, models for these viruses which have similar physicochemical properties must be used.
- Material safety data (MSD) sheets or comprehensive operator manuals must provide information on the following: packaging, chemical composition, storage requirements, stability, handling/disposal and any interfering substances.

## 9 Sample transportation

- The screening procedure must include a means of sample transportation. Details of postal approval must be provided.
- The supplier must provide details of a sample tracking system if it is available.

## 10 Additional requirements

The system should be compatible with requirements for additional tests (e.g. human papillomavirus testing) that may need to be performed on the same sample.

## References


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2. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices (OJ L 331, 7.12.1998).

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4. Control of Substances Hazardous to Health (COSHH) Regulations 2002.

5. Liquid Based Cervical Cytopathology Training Log. NHS Cancer Screening Programmes, 2004.

6. External Quality Assessment Scheme for the Evaluation of the Papanicolaou Staining in Cervical Cytology: Protocol and Standard Operating Procedures. NHS Cancer Screening Programmes, 2004 (NHSCSP Publication No 19 in press).

**Appendix B**

## Patient information sheet

Participant Information Sheet Phase 1 Version 1
REC 07/H1003/109

### PARTICIPANT INFORMATION SHEET

**Evaluation of the potential of second generation Liquid Based Cytology (LBC) techniques for detection of cervical abnormality**

**Invitation to participate**

We would like to invite you to take part in a research study conducted by the Manchester Cytology Centre (MCC). Before you decide whether or not to participate, it is important for you to understand the reasons for the study and what it would involve for you. Please take time to read this leaflet and discuss it with family and friends or your own doctor if you wish. You will also be able to talk to the colposcopist or nurse practitioner on your appointment date about the study and clarify any doubts.

In the meantime if you would like more information please call our independent programme coordinator Mrs Janet Marshall on 0161-276-5103

The leaflet is divided into two (2) parts:

- Part 1 explains the purpose of the research and what will happen if you take part
- Part 2 provides information on data protection and research conduct

## PART 1

**What is the purpose of the study?**
The aim of the study is to compare the performance of a new cervical sampling and processing system to that of an established NHS approved one.

**Why have I been invited?**
You have been invited because you are due to have an examination at colposcopy clinic where routinely you will have a cervical smear test done.

**Do I have to take part?**
It is up to you to decide. We will describe the study as we go through this information sheet and if you do decide to take part we will ask you to sign a consent form to show you fully understand the study and you are in agreement. You are free to withdraw at any time, without giving a reason and this in no way will affect your standard care.

**What is being tested?**
New sampling and processing systems, 2nd Generation Liquid Based Cytology (LBC), with which to take and prepare cervical samples are being tested. Currently in England and Wales there are two systems which have been tested and approved by the NHS Cervical Screening Programme. For any other methods to be approved there must be evidence to show that its performance meets the standards of these systems in terms of clinical usefulness, value for money and acceptance by patients. In this the first phase of the study, the 2nd Generation LBC technology will be assessed against the performance of one that is NHS approved, either ThinPrep or SurePath.

**What will happen to me if I take part?**
When you come to colposcopy clinic on your appointment day, the colposcopist or nurse practitioner will go through the study again with you and answer any questions you may have. If you do agree to take part you will then have an examination and two cervical samples will be taken: one with the NHS approved device, ThinPrep or SurePath and the other with that of the 2nd Generation LBC technology. The order by which the samples will be taken will be random. Both samples will be sent to the Manchester Cytology Centre where assessment will be done and the two methods will be compared.
The time required to have the second sample will be approximately twenty (20) seconds.

**What do I have to do?**
You do not have to do anything different and there are **no** lifestyle, medical health product or dietary restrictions.

**What are the possible disadvantages and risks of taking part?**
Taking a second cervical sample will result in your colposcopy examination lasting approximately twenty seconds more but there should be no additional discomfort. Neither will your health be at risk.

**What are the possible benefits of taking part?**
There are no potential benefits.

**What happens when the research study stops?**
On completion of this phase of the study, the results will be assessed and if the 2nd Generation LBC system compares favourably with the NHS approved method then phase 2 will take place on another patient population.

**Will I be paid?**
No payment will be made

**Will my taking part be kept confidential?**
Yes. We will follow ethical and legal practice and all information will be handled in confidence. All information will be handled in adherence to the Data Protection Act (1998) and Trust Confidentiality policies

**End of Part 1**

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

## PART 2

**What if relevant new information becomes available?**
Sometimes we get new information about devices and tests being studied. If this happens, the colposcopist or nurse practitioner will tell you and discuss whether you should take part in the study.
If at the end of this phase we do not have a favourable performance indicator of the 2nd Generation technology then we will not proceed to phase 2.

**What will happen if I don't want to carry on?**
This phase of the study requires one extra sample to be taken at your colposcopy visit. You are free to decline participation and we would like to assure you that your standard treatment will in no way be affected.

**What if there is a problem?**
Every effort will be made to ensure you fully understand the study and all your concerns addressed during this time. We do not believe you will suffer any harm by participating; however, if you have any complaint about the way you have been dealt with please contact our independent programme coordinator Mrs Janet Marshall on 0161-276-5103. If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure.

**What will happen to any samples I give?**
At the laboratory both samples will be prepared and processed onto glass slides for interpretation by technical and medical staff. In accordance with the Human Tissue Act and the Royal College of Pathologist recommendations, any unused material will be ethically and confidentially disposed after assessment. The slides will be stored for ten years in accordance with the aforementioned recommendations and after such time, these too will be disposed in the same ethical and confidential manner.

**What will happen to the results of the study?**
On completion of the study a written report will be made to the Centre for Evidence Based Purchasing, the commissioners of the study. This report will be available online.

**Who is organising and funding the research?**
The study is organised by the Manchester Cytology Centre under the Guidance of the guidance of Central Manchester and Manchester Children's University Hospitals NHS trust, whilst funding has been provided by the Centre for Evidence Based Purchasing who commissioned the study.

**Who has reviewed the study?**
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the South Manchester Research Ethics Committee

**End of Part 2**

Thank you for taking the time to read this leaflet. If you have any questions please call our independent programme coordinator Mrs Janet Marshall on 0161-276-5103.
At colposcopy you will also have a chance to discuss the study and decide if you would like to take part.

**Appendix C**

## Consent form

Study Number: REC 07/H1003/109

Phase: 1

Patient Identification Number for this Trial:

## CONSENT FORM

**Title of Project:** Evaluation of the potential of second generation Liquid Based Cytology (LBC) techniques for detection of cervical abnormality in a high prevalence setting of colposcopy.

**Name of Lead Researcher**: Dr. Minaxi Desai

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**Please read carefully and initial boxes if in agreement**

**Participant Initials**

1) I confirm I have received in advance, read and understood the Patient Information Sheet (Version 1) for the above study and have had the opportunity to ask questions. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3) I understand that during this study I would have two (2) cervical samples taken, one using an NHS approved sampling device, SurePath or ThinPrep and the other using that of a 2nd Generation LBC Technology. ☐

4) I understand the order by which the smears are taken will be random ☐

5) I understand that sections of my cervical screening records may be looked at by responsible individuals from the Manchester Cytology Centre and only where it is relevant to this study. ☐

6) I agree to take part in this study ☐

| _____ | _____ | _____ |
| --- | --- | --- |
| Name of Patient | Date | Signature |

| _____ | _____ | _____ |
| --- | --- | --- |
| Name of consent taker | Date | Signature |

_____
Designation

1 for patient; 1 for researcher, 1 for patient's notes

**Appendix D**

## Manual staining system

The steps involved in this staining method are shown in the table 11 below.

Table 11: Staining steps involved for Cell Solution 120 samples

| Step No. | Reagent | Time |
|---|---|---|
| 1 | Tap water | 1:00 |
| 2 | Gill's haematoxylin | 4:00 |
| 3 | Tap water | 10 dips |
| 4 | Tap water | 10 dips |
| 5 | 95% alcohol | 10 dips |
| 6 | 95% alcohol | 10 dips |
| 7 | OG-6 | 0:30 |
| 8 | 95% alcohol | 10 dips |
| 9 | 95% alcohol | 10 dips |
| 10 | EA-50 | 6:00 |
| 11 | 95% alcohol | 10 dips |
| 12 | 95% alcohol | 10 dips |
| 13 | 100% alcohol | 10 dips |
| 14 | 100% alcohol | 10 dips |
| 15 | 100% alcohol | 10 dips |
| 16 | Xylene | 1:00 |
| 17 | Xylene | 1:00 |

## Appendix E

### Maintenance sheet for CellSolution 120™

Maintenance

On holidays from 04/12/08 → 01/01/2009

**CellSolution 120 Maintenance Log** Month: December '08

Billion

| Daily | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Remove and Empty Items | V | | | | | | | | | | | | | | | | / | | | | | | | | | | | | | | |
| Cap Fluid Containers | V | | | | | | | | | | | | | | | | / | | | | | | | | | | | | | | |
| Flush GluCyte Lines | V | | | | | | | | | | | | | | | | V | | | | | | | | | | | | | | |
| Flush Preserv. Lines | V | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| Weekly | Week 1 (01/12/08) | Week 2 ( / / ) | Week 3 ( / / ) | Week 4 ( / / ) | Week 5 ( / / ) |
|---|---|---|---|---|---|
| Wipe Slide Trays | V | | | | |
| Clean Rear Catch Pan | V | | | | |
| Wipe Slide Conveyor | V | | | | |
| Wipe Tube Discharge Chute | V | | | | |
| Inspect Water Bottle | V | | | | |
| Inspect Tubing | V | | | | |

| Semi-Annual | ( / / ) |
|---|---|
| Clean Water Bottle | |
| Check Fitting Fightness | |
| Perform Water Pump Calibration Check | |
| Perform GluCyte Pump Calibration Check | |
| Perform Preservative Pump Calibration Check | |
| Perform Pipetter Calibration Check | |
| Perform Ultrasonic Calibration Check | |
| Clean Blower Inlet Filters | |

**Appendix F**

**Data sheets- CEP technical evaluation of LBC systems –**

**1. QC data sheet**

**System X QC data**

This form should be completed for each run.

| **Run date** | **Run number** | **Start time** |
|---|---|---|
| **Finish time** | **No. samples processed** | |

| **Details of any samples not processed*** |
|---|
| |

Macroscopic assessment of slides. (Please assess on overall appearance of run. If individual cases differ significantly record in additional comments section.)

**Amount of material** (please circle one)

**Low** **Average**

**Distribution of material** (circle yes or no and give brief details)

| | |
|---|---|
| **Holes** | Yes / No |
| **Crescents** | Yes / No |
| **Peripheral rim** | Yes / No |
| **Other** | Yes / No |

| **Additional comments*** |
|---|
| |

**Repeat preparations required** **Yes / No** (please circle one)
If yes, please state reason and give details*

## 1. QC data sheet (continued)

| |
|---|
| **Supplemental processing required** **Yes / No** (please circle one)<br>If yes, please state reason and give details* |

*If insufficient space please continue overleaf

**2. CEP technical evaluation of LBC systems – macroscopic/microscopic/reproducibility data sheet**

**System X**

**Run date** **Run number**

**Case number** **Repeat number**

**Macroscopic assessment of cellularity** (circle one choice)

**Low** **Average** **High**

**Macroscopic assessment of evenness of distribution** (lack of holes, crescents etc, if poor state type of unevenness)

| | | | Comment |
|---|---|---|---|
| Good | Average | Poor | |

**Microscopic assessment**

**Cell numbers** (circle one choice, perform cell count if appears <15000)

**<5000 cells** **5000-15000 cells** **>15000 cells**

**Quality of preparation** (circle one choice for each, if a choice with an asterix is circled please specify the cause in the comments box

| | | | | | Comment |
|---|---|---|---|---|---|
| **Cell presentation** | Good | Satisfactory | Poor* | Very poor* | |
| **Cytolysis** | None | Slight | Some | Marked | |
| **Obscuring elements** | None | Some | Moderate* | Marked* | |
| **Cytoplasmic staining** | Good | Satisfactory | Poor* | Very poor* | |
| **Nuclear staining** | Good | Satisfactory | Poor* | Very poor* | |
| **3-dimensionality** | Flat | Slight | Moderate | Marked | |
| **Cell drift** | Present | | Absent | | |

**2. CEP technical evaluation of LBC systems – macroscopic/microscopic/reproducibility datasheet (continued)**

**Repeat/supplemental processing**

| | | | **Details** (if yes) |
|---|---|---|---|
| **Repeat processing required** | Yes | No | |
| **Supplemental processing required** | Yes | No | |

**Cell types present** (circle all seen)

| | | |
|---|---|---|
| Superficials | Intermediates | Parabasal/basal |
| Metaplastics | Endocervicals | Endometrials |

**Dyskaryosis** (state highest grade seen and circle amount)

| | | | |
|---|---|---|---|
| **Grade** | | | |
| **Amount** | Scanty | Some | Many |
| **Specific type(s) if present** | Small cell | Pale cell | Bland cell |
| | Microbiopsy | Other: | |

| **Comments:** |
|---|
| |

**3. System X error log**

| **Run date** | | **Run number** | |
|---|---|---|---|
| **Error logged** (include code) | | | |
| **Remedial action** (say whether engineer called or in-house solution) | | | |
| **Outcome** | | | |
| **Downtime** | | | |

## Appendix G

## Sample Dilution Algorithm

The unit's software uses an algorithm to adjust the amount of cells applied to the slide (cellularity) by varying the amount of water, GluCyte, and transfer volumes for each individual sample. This dilution process is based on an approximation of the number of cells starting in the pelletized sample.

While the software determines the dilution and transfer volumes to arrive at a specific cellularity, it is instructional to examine an example calculation that does the reverse. The following example determines cellularity with assumed volumes as a way to illustrate the equations used in the algorithm.

1. The volume of the cell pellet in the primary tube is determined by translating the pellet height found by the ultrasonic sensor to a volume based on the internal geometry of the tube. In our example assume this volume to be:

V pellet = 100 ul

2. Assume we know that a cell pellet after centrifugation has a concentration of:

C pellet = 21,000 cells / ul

3. The number of cells in the pellet is:

N pellet = C pellet V pellet

= (21,000 cells / ul) 100 ul

= 21,00,000 cells

4. Assume we add 200 ul of water to the tube: V water = 200 ul

5. The volume in the primary tube is now:

V primary = V water V pellet =200 ul100 ul=300 ul

6. Since we know the primary diluted volume in the tube and the number of cells in the tube we can calculate the diluted concentration in the primary tube:

C primary = N pellet / V primary

= 2,100,000 cells / 300 ul

= 7000 cells / ul

7. Assume we transfer 80 ml of fluid out of the primary tube to the secondary tube:

V primary transfer = 80 ul

8. As long as the solution in the primary tube is adequately mixed, the 80 ml aspirated from the solution will have the same concentration as the rest of the solution. We therefore can determine the number of cells carried in the primary transfer volume to be:

N primary transfer = C primary V primary transfer

N primary transfer = 7000 ul x 80 ul = 560,000 cells

9. Assume the volume of GluCyte dispensed into the secondary tube is:

V GluCyte = 200 ul

10. Since the 80 ml of primary transfer solution is added to the secondary tube along with the GluCyte, the total volume of the secondary tube is now:

V secondary =V GluCyte V primary transfer =200 ul  80 ul = 280 ul

11. We know that the 80 ml of primary transfer solution carried 560,000 cells, so we can calculate the resultant concentration in the secondary tube:

C secondary = N primary transfer / V secondary

= 560,000 cells / 280 ul

=  2000 cells / ul

12. Assume we transfer 40 ml of fluid from the secondary tube to the slide:

V secondary transfer = 40ml

13. As long as the solution in the secondary tube is adequately mixed, the 40 ml aspirated from the secondary tube will have the same concentration as the rest of the fluid in the tube. We therefore can calculate the number of cells carried in the slide transfer volume:

N slide = C secondary V secondary transfer

= 2000 ml x 40 ul = 80,000 cells

14. Assume the unit is set up to dispense fluid to the slide in a 15 mm by 20 mm rectangle. This gives an area of:

Area = 15 mm 20 mm= 300 $mm^2$

15. Knowing the number of cells and the deposit area the cellularity is found to be:

Cellularity = N secondary transfer / Area

= 80,000 cells / 300 $mm^2$

= 267 cells / $mm^2$

The above calculation can very precisely determine cellularity so long as the starting pellet volume and pellet concentration are known and the solutions in the primary and secondary tubes are well mixed. While this method produces very consistent cellularity, it is not exact due to sample to sample variations that can cause minor differences in the number of cells per slide.