

## RESEARCH ARTICLE

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# Assessment of PathTezt® Infinity Processor and Autoloader Efficiency in Cervical Smear Analysis

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## Abstract

**Objective:** To assess the cellularity, epithelial cell coverage, cellular arrangement, preservation, and interfering factors of cervical cytological smears produced by the PathTezt® Infinity with Autoloader. **Methodology:** A total of 1003 Pap smear samples were taken from Hospital Pakar Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II and processed using the PathTezt® Infinity. The slides were evaluated for smear adequacy, epithelial cells coverage, cellular arrangement, interfering factors, and cellular preservation. **Results:** Approximately 946 samples (94.1%) were adequate for evaluation. The evaluation of cellular arrangement demonstrated good dispersion in 860 samples (85.7%), while 94.7% of the samples exhibited minimal to no obscuration by inflammatory cells. The presence of erythrocytes did not impede the evaluation of squamous cells in 1002 smears (99.9%). All smears showed good-quality fixation features for nuclear, cytoplasmic, and microorganism evaluation. The overall “good” performance rate was 97.7%. Chi-square analysis between the scoring categories and criteria for scoring showed statistical significance ( $p < 0.001$ ). **Conclusion:** The PathTezt® Infinity with Autoloader produces high-quality smears, is easy to operate, fully walk-away, and cost-effective, making it suitable for cervical cancer screening.

**Keywords:** Liquid-based cervical smear- cost effectiveness- Path Tezt Processor

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## Introduction

Cervical cancer is a serious public health problem worldwide ranked as the third most frequent cancer among women. An estimated 660,000 women diagnosed and 350,000 fatalities expected in 2022 [1] Malaysia has an age-standardized cervical cancer incidence rate of 6.0 per 100,000 women [2], which is lower than the global average of 14.1 per 100,000 but higher than the rate in high-income nations, which is 7.5 per 100,000.

Malaysia has introduced cervical cancer screening programs, mostly based on the Pap smear test, which is intended to detect pre-cancerous and malignant changes early. The Pap smear was launched in Malaysia in 1969 and is now advised for all sexually active women aged 20 to 65 [3]. Initial screens should be performed once a year for two years in a row, and if the findings are normal, future screenings should be performed every three years.

The Malaysian Ministry of Health is working to achieve the World Health Organization's (WHO) strategic target of screening 70% of eligible women at least twice in their lifetime with HPV testing [3]. These measures, together with immunisation campaigns, are critical for lowering cervical cancer incidence and mortality rates.

In Malaysia, the most popular liquid-based cytology (LBC) system used for cervical cancer screening is ThinPrep®. Another common option is SurePath™, but ThinPrep® is more widely recognized due to its early adoption and broader availability in both private and some public healthcare facilities. ThinPrep® uses filter-based concentration technique as its principal method while Sure Path uses the cell enrichment method [4, 5].

The PathTezt® Liquid Based Cytology (LBC) System for cervical smear screening has been in practice in Malaysia for 13 years, making it a second generation of liquid-based preparation (LBP) cytology. Many innovations and improvements have been made over the years to guarantee that the quality of cervical smears generated is always on par with the predecessor. The PathTezt® Infinity with Autoloader (PTI) was created in response to the increased use of the PathTezt® Liquid Based Pap Test. The PathTezt® Infinity with Autoloader (PTI) is a next-generation liquid-based cytology technology that gives PathTezt® cytology specimens with random access and fully walkaway automated processing. The processor can automatically process up to 120 cervical cytology specimens per batch and enables continuous random specimen loading [6]. A previous study that was

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done on the PathTezt® 2000 Processor has proven that the system is reliable and cost-effective for the user [7].

#### Objective

This study is done to evaluate the cellular preservation, morphology, and quality of smear in gynaec cytology as well as diagnostic interpretation of cervical cytological smear produced by PathTezt® Infinity liquid-based processor.

#### Materials and Methods

A total of one thousand and three (1003) samples were obtained from the pool of residual specimens from Hospital Pakar Universiti Sains Malaysia (HPUSM) and Hospital Raja Perempuan Zainab II (HRPZII) in Kota Bharu, Kelantan. Two hundred and twenty-seven (227) were from HPUSM and the rest seven hundred and seventy-six (776) were from HRPZII. The inclusion criteria were all samples must be taken from the cervix area only, not from the vagina area or post-hysterectomy patient. Smears from pregnant women were also excluded from the study. To ensure the integrity and diagnostic utility of cellular specimens derived from residual samples, stringent pre-analytical criteria must be observed. Specifically, the temporal window between specimen collection and processing must not exceed six weeks, or alternatively, samples must be maintained within a controlled temperature range of 15°C to 30°C. These parameters are critical for preserving cellular morphology and viability, thereby minimizing artifacts that could compromise downstream analysis.

Samples originating from HRPZII, initially preserved in SurePath™ solution, undergo a preliminary centrifugation step. This process facilitates the concentration of cellular components, resulting in the formation of a cell pellet. This pellet is then carefully resuspended in PathTezt Preserv Cell Solution, a specialized medium designed to maintain cellular integrity during subsequent processing.

Following this, the samples are subjected to a minimum one-week incubation period within PathTezt® Liquid Based Preparation solution. This incubation period allows for optimal cellular fixation and stabilization, crucial for producing high-quality smears.

The prepared samples are subsequently processed utilizing the PathTezt® Infinity Processor with Autoloader. This automated platform employs a filter-based concentration methodology, a technique that selectively captures and concentrates cellular material while eliminating extraneous debris. This principle is analogous to the established filter-based concentration approach utilized by the PathTezt® 2000 Processor, ensuring a consistent and standardized approach to sample preparation. This type of methodology is important because it provides a monolayer of cells, that can be more accurately analysed, than a traditional smear.

A filter-based concentration technique was employed, comprising vortexing the sample for cell dispersion, followed by collection of the dispersed cells onto a filter via vacuum suction. The collected cells were subsequently transferred from the filter to a glass slide and immediately fixed in an alcohol bath [8]. Following fixation, Papanicolaou staining was performed using the regressive method. Smears were then evaluated by certified Cytoscreeners, with subsequent verification by a Cytopathologist. The analysis focused on sample adequacy, epithelial cells coverage on smear (percentage of the circle), cellular distribution, obscuring factors, and cell fixation. Sample adequacy was assessed in accordance with the guidelines published in The Bethesda System for Reporting Cervical Cytology (2014). Meanwhile the epithelial cells coverage on smear was based on diagram made by Dr. Euphemia McGoogan, a senior lecturer in Pathology from the University of Edinburg, (4) (Figure 1). The study model was based on the previous study on PathTezt® 2000 Processor Liquid Based Cytology System [7, 9].

Cellular arrangement was analysed by assessing the

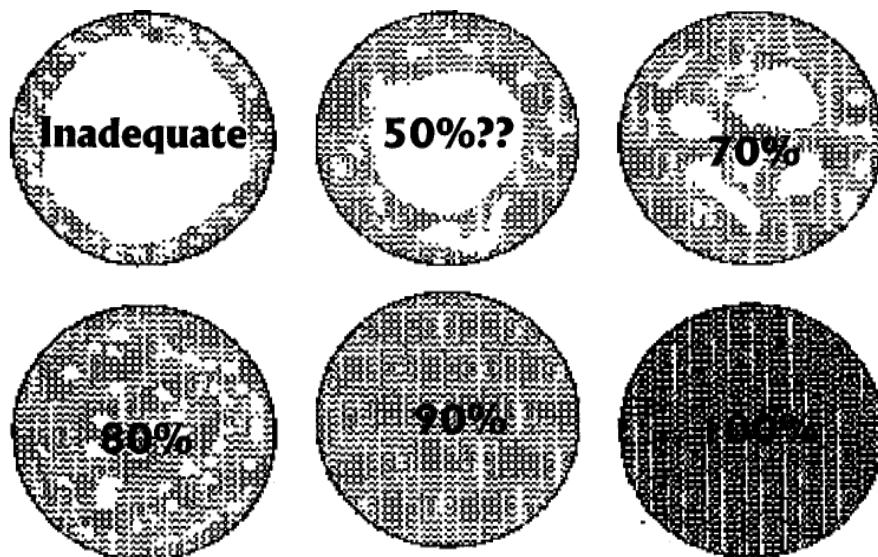


Figure 1. Illustrated Images of the Epithelial Cell's Coverage on Smear. Inadequate to 50% coverage score as 1, 70% to 80% coverage score as 2 and 90% to 100% coverage score as 3. (4)

uniformity of cell dispersion and the degree of cellular overlap on the smear. The presence of interfering factors was quantified by estimating the percentage of the smear area obscured by red blood cells and inflammatory cells. Each of these variables was assigned a score of 1 (poor), 2 (average), or 3 (good). (Figure 2) Cellular preservation quality was evaluated based on the preservation of key cellular and organismal features, including nuclear morphology, cytoplasmic integrity, and the structural integrity of any observed organisms (bacteria, fungi, and protozoa). For these categories, the scoring was either 1 (poor) or 2 (good). The cumulative score for each smear was then classified as poor ( $\leq 9$ ), average (10-15), or good (16-20). The overall performance of the system was determined by calculating the percentage of smears exhibiting a “good” score [9].

#### Statistical analysis

The chi-square test was used to compare the relationship between total score versus criteria for scoring and data agreement in between previously reported smear on Sure Path™ and current smear (PTI) interpretation. The association was determined using multiple logistic regression tests. All calculations were performed by using SPSS version 29. P-value  $< 0.05$  was taken as statistically significant.

## Results

A total of 1003 samples are subjected to this study. The age range was 19-78 years (mean age 39.2+/- and medial age of 43). The most common age group come for pap smear screening are from 30 to 39 years old. Nine hundred and forty-six (94.1%) samples are adequate for interpretation.

Analysis of cellular coverage revealed that 57 (5.6%) samples were interpret as inadequate for interpretation due to insufficient cell representation ( $<50\%$ ). Most samples, 634 (63.2%) exhibited cell coverage within the 60-80% range, while 315 (31.4%) demonstrated near-complete to complete coverage (90-100%) (Table 1 and Figure 1 & 3). During the initial phase of the study, 29 samples were not detected during processing, and one smear was irreparably damaged post-processing, contributing to a technical error rate of 2.92%. This number did not include in total samples. Thus, it were not altered the findings of this study. Evaluation of cellular arrangement demonstrated good dispersion in 860 (85.7%) samples. Squamous epithelial cells were observed in both single and syncytial arrangements. Localized areas of cellular crowding and overlapping, indicative of uneven distribution, were noted in 137 (13.7%) samples. Extensive or pervasive cellular crowding and overlapping were observed in 6 (0.6%) samples (Table 1).

Microscopic analysis of interfering factors revealed that in 3 (0.3%) samples, inflammatory cells obscured greater than 75% of epithelial cells. Partial obscuration (50-70%) was observed in 50 (5%) samples, while 950 (94.7%) samples exhibited minimal to no obscuration by inflammatory cells. Erythrocyte presence did not impede squamous cell evaluation in 1002 (99.9%) of the analysed smears. All smears show 100% cellular preservation despite changing from previous preservative (SurePath™) to PathTezt® for processing purposes (Table 1).

Cytological assessment indicated a negative result for intraepithelial lesion or malignancy (NILM) in 783 (78%) samples. Infectious agents, including bacterial vaginosis, *Actinomyces* sp., and *Candida* spp., were identified in 146 (14.5%) samples. Seventeen (1.7%) cases were classified as abnormal, comprising atypical

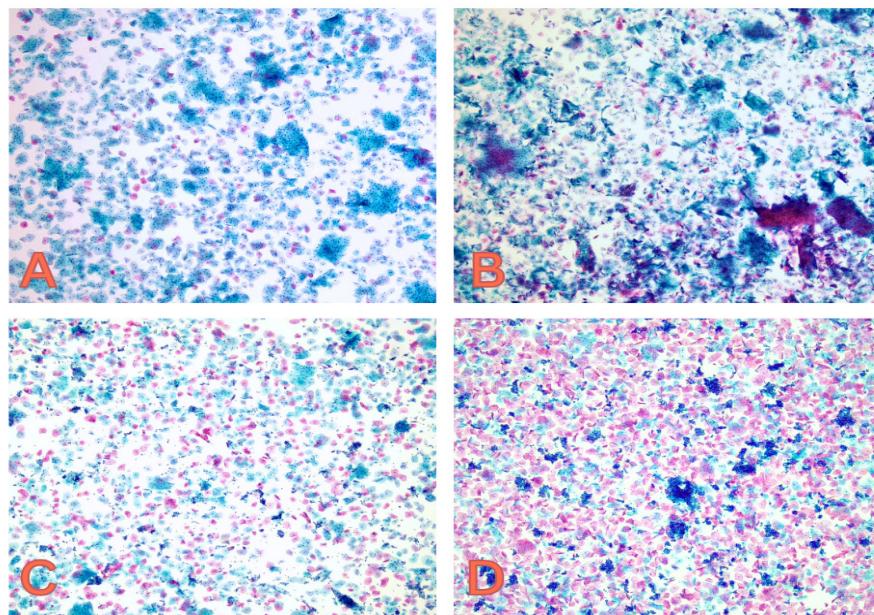


Figure 2. Pap stain x 4 Magnification: Image of the cervical smears showing different appearance of the cells for cellular arrangement and obscuring factor by inflammatory cells. A) Cells are not evenly scattered. The cells are crowded and overlapping some areas. Score 2 (Average). B) Cells are not evenly scattered. The cells are crowded and overlapping in most areas. Score 1 (Poor). C) Inflammatory cells are not obscuring the squamous cells. Score 3 (Good). D) Inflammatory cells are partially obscuring (50 to 70%) of the squamous cells. Score 2 (Average).

Table 1. Evaluation Score and Percentage

Criteria	Categories	Score	n (%)
I) Smear Adequacy	Inadequate cellularity	1	57 (5.7%)
	Adequate cellularity	2	946 (94.3%)
ii) Epithelial cells coverage	Below 50% coverage	1	54 (5.4%)
	60% to 80% coverage	2	634 (63.2%)
	90% to 100% coverage	3	315 (31.4%)
iii) Cellular Arrangement	Poor (1 mark)	1	6 (0.7%)
	Average (1 mark)	2	136 (13.6%)
	Good (1 mark)	3	860 (85.7%)
iv) Interfering factor	Presence of inflammatory cells.		
	Poor (1 mark)	1	3 (0.3%)
	Average (1 mark)	2	50 (5%)
	Good (1 mark)	3	950 (94.7%)
	Presence of red blood cells (RBCs).		
	Poor (1 mark)	1	0 (0%)
	Average (1 mark)	2	2 (0.1%)
v) Cellular preservation	Good (1 mark)	3	1001 (99.9%)
	Nuclear features		
	Poor (1 mark)	1	0 (0%)
	Good (1 mark)	2	1003 (100%)
v) Cellular preservation	Cytoplasmic features		
	Poor (1 mark)	1	0 (0%)
	Good (1 mark)	2	1003 (100%)
v) Cellular preservation	Organisms (Bacteria, fungal and protozoa).		
	Poor (1 mark)	1	0 (0%)
	Good (1 mark)	2	1003 (100%)
Total score	Poor (1 mark)	1 to 9	0 (0%)
	Average (1 mark)	10 to 15	3 (0.3%)
	Good (1 mark)	16 to 20	1000 (97.7%)

squamous cells of undetermined significance (ASC-US, n=3), atypical squamous cells, cannot exclude high-grade (ASC-H, n=1), low-grade squamous intraepithelial lesion (LSIL, n=4), high-grade squamous intraepithelial lesion (HSIL, n=1), HSIL with suspected invasion (n=1), squamous cell carcinoma (n=1), atypical glandular cells (AGC) endocervical (n=1), AGC endometrial (n=1), AGC glandular (n=2), AGC favour neoplastic (n=1), and

adenocarcinoma of the endometrium (n=1). (Figure 4) Comparison with prior cytological diagnoses yielded a discordance rate of 7.32%. Chi-square analysis confirmed a statistically significant agreement between the current smear and the previous smear diagnosis ( $p < 0.05$ ) (Table 2).

A majority of the evaluated smears (97.7%) demonstrated “good” performance, as defined by scores

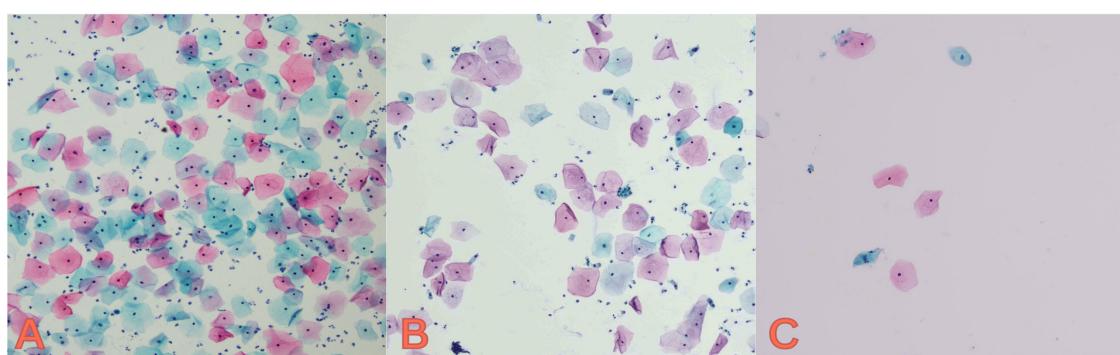


Figure 3: Pap stain x 10 magnification Image of the smears showing cellularity adequacy based on epithelial cells coverage. A. Adequate cellularity with 90 to 100% epithelial cells. B. Adequate cellularity with 60 to 80% of epithelial cells coverage. C. Inadequate cellularity with less than 50% of epithelial cells coverage.

Table 2. The Correlation between PTI Smear Interpretation and Previous Smear (Surepath™) Interpretation

	PTI Smear Interpretation	Previous Smear (Surepath™) Interpretation						Total
		Unsatisfactory for evaluation	NILM	Infection	Squamous Intraepithelial Lesion (SIL) or Atypical Glandular Cells (AGC)	Malignant	Total	
PTI Smear Interpretation	Unsatisfactory for evaluation	9	40	7	1	0	0	57
	NILM	0	778	2	3	0	0	783
	Infection	0	1	145	0	0	0	146
	Squamous Intraepithelial Lesion (SIL) or Atypical Glandular Cells (AGC)	0	1	0	14	0	0	15
	Malignant	0	0	0	0	2	2	2
	Total	9	820	154	18	2	2	1003

The result is significant at  $p < 0.001$  using Pearson Chi-square test.

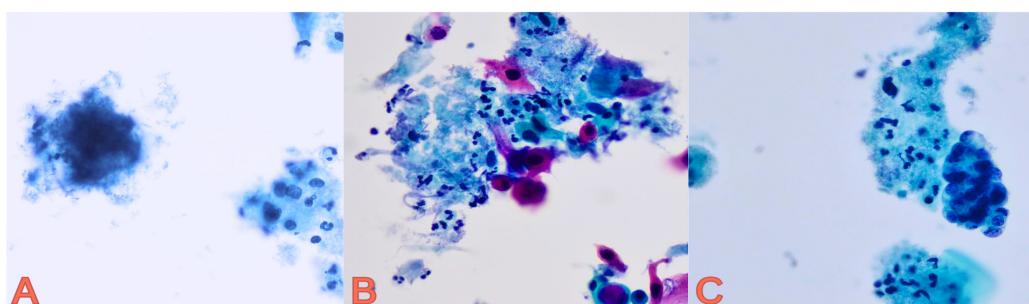


Figure 4. Pap Stain x 40 Magnification. Images of the diagnostic cells taken from the study. A. Actinomyces spp. organism. B. Squamous cell carcinoma (SCC). C. Adenocarcinoma of endometrial cells.

within the 16 to 20 range. A small fraction (0.3%, n=3) exhibited “average” performance (scores 10 to 15), while no smears rated as poor performance. A statistically significant relationship was observed between the total performance score and the individual scoring criteria ( $p < 0.05$ ), indicating that sample adequacy, epithelial cell coverage, cellular distribution, and the presence of obscuring factors (both inflammatory cells and erythrocytes) significantly influenced the overall performance rating (Table 3).

## Discussion

Cervical cancer is the third most prevalent malignancy among females. In most age groups, the incidence rate is lower in 2017 to 2021 than it was in 2012 to 2016.

Table 3. The Correlation between PTI Smear Interpretation, and Total Score Versus Age and Criteria for Scoring

Variables	PTI Smear Interpretation	Total score
Age	0.01	0.418
Smear Adequacy	<0.001	<0.001
Epithelial Cells Coverage	<0.001	0.003
Cellular Arrangement	0.371	0.39
Interfering Factor: Inflammation	<0.001	<0.001
Interfering Factor: Red Blood Cells	0.732	0.938
Total Score	<0.001	N/A

+P-value < 0.05 is significant. \*Analysis is done using Pearson Chi Square tests.

Despite a slight decrease in incidence from 2017 to 2021, cervical cancer risk remains highest for women aged 75 and above (21.7%), with the 60-64 age group also at significant risk (20.7%). The incidence rate for cervical cancer in women fell from 6.2 to 6.0 during this period. (2) The Global Cancer Observatory (GLOBOCAN) data 2022 shows that the incidence rate and mortality rate for cervical uteri cancer are 12.1% and 19.3% from overall cancer, respectively [10].

Cervical cancer is highly preventable and treatable, especially because it often progresses slowly when detected early. The causal link between HPV and cervical cancer has resulted in the development of methods that can be utilised to eliminate cervical cancer [3]. Regular cervical cytology screening (Pap smear) is a widely recognized and effective method for cervical cancer prevention. Current guidelines generally recommend annual screening or triennial screening following two consecutive negative results. Women who do not adhere to recommended screening schedules experience a substantially increased risk of developing cervical cancer, with estimates suggesting a 3- to 10-fold higher risk compared to regularly screened individuals [11]. Epidemiological data indicate that cervical cancer is infrequent in women under 25 years of age. Furthermore, cervical changes are common in this age group, which reduces the cost-effectiveness of routine screening in this population [12]. In 2010, Malaysia implemented HPV vaccination within its National Immunization Programme. This implementation was informed by cost-effectiveness analyses conducted at both national and international levels [13]. The National HPV Immunization Programme

targets female students aged 13 years for vaccination, utilizing a school-based health service delivery model [2]. Malaysia has adopted HPV screening test as primary screening publicly in 2015 which targeted to sexually active women aged 30 to 65 years. Women younger than 30 years has been offered to do cytology screening instead (conventional / liquid-based cytology) during their visit on post-natal clinic [14].

Liquid-based preparation (LBP) surpasses conventional smears by eliminating obscuring debris, like red blood cells and mucus, and producing a uniform, thin-layer cell monolayer, thereby enhancing cellular visualization and screening efficiency [4]. The PathTezt® Infinity with Autoloader (PTI) represents a relatively recent introduction to the market. Consequently, comparative studies evaluating its performance against established methodologies are currently limited. This study aims to document both favorable and less favorable findings observed during initial implementation.

Key advantages of the PTI include significant improvements in processing throughput, enabling the rapid processing of high sample volumes. The system's fully automated, 'walk-away' operation substantially reduces technical staff processing time, leading to enhanced economic efficiency and optimized staff management. Furthermore, the PTI's random access and STAT prioritization capabilities facilitate improved laboratory turnaround times for urgent cases. This feature allows for the interruption of batch processing to accommodate immediate processing of critical samples.

According to the Bethesda System for Reporting Cervical Cytology (2014), a minimum of 5,000 squamous or squamous metaplastic cells is recommended for adequate evaluation of liquid-based cytology specimens. However, acknowledging the potential for reduced cellularity in certain patient demographics, the system permits laboratory discretion in establishing adequacy criteria. These demographics include individuals undergoing chemo/radiotherapy, post-menopausal women exhibiting atrophic changes, and women post-hysterectomy [15].

This study result shows quite a minor percentage of unsatisfactory smears. The main reason for the unsatisfactory smear from this study was due to scanty squamous cell components. This may be due to the inconsistency volume of residual samples used for the study during the earlier period. There is also an incidence where the smear did not adhere properly to the slides after processing in PT Infinity Autoloader. The main cause for lack of adherence can be due to lack of preserving time from the cell pallets into the PathTezt® preserve solution. Percentage of circle covered by epithelial cells are commensurate with the adequacy of the samples because the lower the percentage is, the fewer cells adhere to the slide.

In the initial phase of the study, a technical issue arose when the equipment failed to detect samples. This was traced to a misaligned specimen track, a problem that originated during the equipment's delivery from the manufacturer, preventing the sensor from functioning correctly. In addition to the detection failures, this

equipment issue led to a processed slide being broken beyond repair. However, this problem was solved after the manufacturer came and identified the issues.

The cellular arrangement of the smears from this study also shows an admirable result. Only 0.7% of the smears having cells that are not evenly scattered, crowded, and overlapping in most areas, this only shows that the smears are of high quality in terms of cellular distribution when compared to conventional smears. The PathTezt® system relies on the principles of dispersion and vacuum techniques. These techniques contribute to the system's efficacy by minimizing obscuring factors such as mucus, inflammatory cells, and erythrocytes. The presence of inflammatory cells serves as a marker for potential infection or other, currently unidentified, pathological processes. Statistical analysis demonstrated minimal background interference, with only a small proportion of smears exhibiting >75% coverage by inflammatory cells or erythrocytes. This indicates effective erythrocyte lysis during processing, a key advantage of liquid-based cytology over conventional smears.

Results indicate that transitioning from alternative preservative or liquid-based preparation solutions does not compromise cellular preservation. The PathTezt® methanol-based solution effectively maintains cellular integrity, exhibiting excellent compatibility with Papanicolaou staining. This results in clear nuclear detail and readily discernible cytoplasmic features. Infective microorganism, pre-cancerous cells and cancerous cells are easily seen under microscopic examination. Figure 4 shows selected cell images taken from the slides of this study. Nuclear details and cytoplasmic differentiation can be appreciated easily.

Smear quality, determined by sample adequacy, cellular distribution, cell coverage, and the absence of obscuring factors like inflammatory cells and erythrocytes, is crucial for accurate cytopathological diagnosis and subsequent patient management. This fact is evidenced by the correlation analysis that was done which shows a significant p-value of < 0.001. Table 3 shows the correlation between PTI smear interpretation and total score versus age and criteria for scoring.

In conclusion, comparative evaluations of cervical screening systems are inherently susceptible to bias. While a split-sample study design was implemented, its limitations became apparent. Specifically, the sequential processing of PathTezt® samples introduced a potential bias, as they were consistently the second smear prepared. This factor compromises the direct comparison of sensitivity and specificity between the techniques. Furthermore, there is a paucity of research focused solely on slide quality as a primary evaluation criterion.

Despite these limitations, our findings demonstrate that the PathTezt® Infinity with Autoloader produces liquid-based cytology (LBC) slides of equivalent quality to existing LBC systems. Moreover, this system offers significant advantages in terms of increased throughput and automated workflow, particularly beneficial for high-volume laboratories.

## Author Contribution Statement

Nur Syuhada ; draft the manuscript , Siti Azrin ; helping in statistical analysis and Anani Aila ; proof of the study finding and manuscript draft.

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### Conflict of interest

None.

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