Quantitative cytomorphological comparison of SurePath and ThinPrep liquid-based cytology using high-grade urothelial carcinoma cells

Okuda, Chihiro ; Kyotake, Aiko ; Nakamura, Akihiro ; Itoh, Tomoo ; Kamoshida, Shingo ; Ohsaki, Hiroyuki

(Citation)
Cytopathology,32(5):654-659

(Issue Date)
2021-09

(Resource Type)
journal article

(Version)
Accepted Manuscript

Quantitative cytomorphologic comparison of SurePath and ThinPrep liquid-based cytology using high-grade urothelial carcinoma cells

Running title: Comparison of SurePath and ThinPrep

Manuscript word: 2131

Table: 1

Figure: 8

Chihiro Okuda[1], Aiko Kyoutake[2], Akihiro Nakamura[3], Tomoo Itoh[2], Shingo Kamoshida[1], and Hiroyuki Ohsaki[1]

[1]Department of Medical Biophysics, Kobe University Graduate School of Health Sciences, Kobe, Japan

[2]Department of Diagnostic Pathology, Kobe University Graduate School of Medicine, Kobe, Japan

[3]Department of Clinical Laboratory Science, Faculty of Health Care, Tenri Health Care University, Tenri, Japan

Corresponding author: Hiroyuki Ohsaki, PhD

Department of Medical Biophysics, Kobe University Graduate School of Health Sciences

7-10-2 Tomogaoka, Suma-ku, Kobe, 654-0142, Japan

Tel: +81-78-796-4591

Email: ohsaki@people.kobe-u.ac.jp

Conflict of interest disclosures: The authors have no conflicts of interest to declare.

Funding support: Dr. Hiroyuki Ohsaki was supported by a grant from The Japanese Society of Clinical Cytology.

Abstract

Objective: In The Paris System for Reporting Urinary Cytology (TPS), the important cytomorphologic features for diagnosing high-grade urothelial carcinoma (HGUC) are a nuclear-to-cytoplasmic (N:C) ratio exceeding 0.7, hyperchromasia, coarse chromatin, and irregular nuclear borders. However, the quantitative cytomorphologic assessment of HGUC cells using SurePath slides is rare. Therefore, we evaluated HGUC cells on SurePath slides quantitatively using a digital image analysis system and compared these data with ThinPrep data.

Methods: The same urine samples were divided into two aliquots and used to prepare SurePath and ThinPrep slides. We used ImageJ to measure the N:C ratio, hyperchromasia, and irregular nuclear borders for HGUC cells on SurePath and ThinPrep slides.

Results: The total number of analyzed HGUC cells on SurePath slides was 981, versus 889 on ThinPrep slides. Hyperchromasia and irregular nuclear borders were significantly more severe on SurePath slides than on ThinPrep slides. Conversely, the N:C ratio did not differ between the methods. Additionally, HGUC cells with N:C ratios exceeding 0.7 were present on almost all slides for both methods.

Conclusions: Our data indicated the reasonableness of using the N:C ratio as the major criterion for the TPS on both SurePath and ThinPrep slides, and an N:C ratio cutoff of 0.7

was suitable for identifying HGUC cells. Meanwhile, the severity of hyperchromasia and irregular nuclear borders differs between the processing methods.

Key words: urine cytology, high-grade urothelial carcinoma, SurePath, ThinPrep, liquid-based cytology, The Paris System for Reporting Urinary Cytology

INTRODUCTION

Urothelial carcinoma is the most common malignancy of the urinary tract, including the bladder, renal pelvis, and ureter. Since urine cytology is noninvasive and inexpensive, it has been widely used for the diagnosis and follow-up of urothelial carcinoma.[1-3] Additionally, the advantages of urine cytology include its high sensitivity and specificity for high-grade urothelial carcinoma (HGUC). Conversely, the low sensitivity and specificity of urine cytology for low-grade urothelial carcinoma are problematic.[3,4] Therefore, The Paris System for Reporting Urinary Cytology (TPS) was published in 2016 as a standardized reporting system for detecting HGUC. According to the TPS, the important cytomorphologic features for diagnosing HGUC are a nuclear-to-cytoplasmic (N:C) ratio greater than 0.7, moderate-to-severe hyperchromasia, coarse chromatin, and markedly irregular nuclear borders. These morphologic features must be present in at least 5–10 well-preserved abnormal cells on slides.[5-7]

These cytomorphologic criteria were derived from studies using liquid-based cytology (LBC) performed with ThinPrep (Hologic, Marlborough, MA, USA) and SurePath slides (Becton-Dickinson, Franklin Lakes, NJ, USA).[8] In recent years, comparative studies of the ThinPrep and Cytospin methods have assessed diagnostic accuracy and quantitative cytomorphology based on the TPS and reported no significant differences.[4,8] Although

several studies compared the conventional method with ThinPrep or SurePath, few studies compared ThinPrep and SurePath using non-gynecological specimens.[9-11] ThinPrep and SurePath are both classified as LBC methods, though they are likely to be different cytomorphologically because they have different principles. To our knowledge, quantitative cytomorphologic data on HGUC cells using SurePath slides have rarely been reported since the TPS criteria were published.[12]

To clarify the cytomorphologic features of HGUC cells on SurePath slides, we evaluated these cells quantitatively regarding the N:C ratio, hyperchromasia, and irregular nuclear borders using a digital image analysis system. Furthermore, these data were compared with ThinPrep data.

MATERIALS AND METHODS

Patients and urine samples

This study used the urine of 13 patients (10 males and 3 females, mean age = 75.2 ± 6.7 years) histopathologically diagnosed with HGUC using biopsy or surgical specimens in Kobe University Hospital.

All samples were residual urine (> 20 mL) obtained after routine urine cytology. After thorough stirring, the same samples were divided into two aliquots and used to prepare

SurePath and ThinPrep slides. There were more than 10 HGUC cells on all SurePath and ThinPrep slides.

SurePath and ThinPrep slides

SurePath slides were prepared following modified manual protocols.[13] Briefly, urine was centrifuged at 3000 rpm for 2 min. The sediment was resuspended in 10 mL of CytoRich Red (Becton-Dickinson). After a 30-min fixation, the specimen was centrifuged. Six milliliters of distilled water were added to the sediment, and the specimen was centrifuged. Another 300 μL of distilled water were added to the sediment, and the specimen was resuspended. Then, the specimen was transferred into the settling chamber (Becton-Dickinson) and mounted on the positively charged slide (Becton-Dickinson) for 15 min. The slide rack (Becton-Dickinson) was then turned upside down to discard the supernatant, and the inside of the settling chamber was rinsed with 95% alcohol. After discarding the 95% alcohol, the settling chamber was removed, and the slide was immediately placed in 95% alcohol.

ThinPrep slides were prepared using the ThinPrep 5000 automated slide processor (Hologic).

Digital image analysis

Images of HGUC cells on SurePath and ThinPrep slides were captured by a single

cytotechnologist (HO) using a microscope at an objective magnification of ×100 and cellSence software (Olympus Corporation, Tokyo, Japan). The criteria for selecting HGUC cells were clear borders for the cytoplasmic membrane and nuclear membrane. Therefore, HGUC cells with unclear or overlapping borders were excluded from this study. A maximum of 100 HGUC cells were evaluated on SurePath and ThinPrep slides; if fewer than 100 cells were present, then all cells on the slide were evaluated.

For digital image analysis, we used ImageJ software (National Institutes of Health, Bethesda, MD, USA), and images were handled as red–green–blue images with 8 bits of resolution. The boundary between the cytoplasm and nucleus was manually traced for all HGUC cell images using XP-Pen Star G640 (XP-Pen, Shenzhen, China) by a single medical technologist (CO) (Figure 1). Using the ImageJ measuring tool, the cytoplasmic area, nuclear area, nuclear mean gray value, and nuclear roundness were obtained. The N:C ratio was calculated by dividing the nuclear area by the cytoplasmic area. The nuclear mean gray value and nuclear roundness were surrogates for hyperchromasia and irregular nuclear borders. Additionally, referring to a previous study [8], we selected the five HGUC cells with the largest N:C ratios on the slide to analyze the N:C ratio and nuclear mean gray value.

Statistical analysis

The Mann–Whitney *U* test was used where appropriate. $p < 0.05$ indicated statistical significance. All analyses were performed using StatFlex software (version 7.0; Artec Inc., Osaka, Japan).

Ethical considerations

This study was approved by the ethics committee of the Kobe University Graduate School of Medicine (No. B190148) and conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

The number of HGUC cells analyzed

The total number of analyzed HGUC cells on SurePath slides was 981 (mean= 75.5, range = 17–100) in 13 cases, versus ThinPrep slides was 889 (mean = 68.4, range = 13–100).

Nuclear and cytoplasmic areas

The mean nuclear and cytoplasmic areas were significantly lower on SurePath slides than on ThinPrep slides (both $p < 0.001$, Table 1).

N: C ratio

The N:C ratio did not significantly differ between SurePath and ThinPrep slides for

both all cells (0.54 vs. 0.54) and the five cells with the largest N:C ratios (0.72 vs. 0.71) (Figure 2).

Nuclear mean gray value (Hyperchromasia)

According to the nuclear mean gray value, nuclear hyperchromasia was significantly more severe on SurePath slides than on ThinPrep slides for both all cells (93.9 vs. 110.8) and five cells with the largest N:C ratios (97.6 vs. 119.2) (both $p < 0.001$) (Figure 3).

Nuclear roundness (Irregular nuclear borders)

As a surrogate for irregular nuclear borders, the mean nuclear roundness was significantly higher on ThinPrep slides than on SurePath slides ($p = 0.024$), indicating that irregular nuclear borders were more prominent on SurePath slides (Figure 4).

DISCUSSION

The present study revealed that the nuclear area, cytoplasmic area, nuclear mean gray value (hyperchromasia), and roundness (irregular nuclear borders) of HGUC cells were significantly different between SurePath and ThinPrep slides. Conversely, there was no significant difference in the N:C ratio. Likewise, in the analysis of the five cells with the largest N:C ratios, the nuclear mean gray value was significantly different between

SurePath and ThinPrep slides, although the N:C ratio did not differ.

The principles of the SurePath method are gravity sedimentation and electrical adhesion.[13] The sample is transferred into the settling chamber, mounted on the positively charged slide, and allowed to stand. During the interval, cells are sedimented via gravity on the slide, and because cells are negatively charged, they electrically adhere to the slide. In the SurePath method, cells are always immersed in fluid; therefore, drying is avoided. In addition, cells are smeared via gravity sedimentation, and they are not physically stimulated. Consequently, cells retain their original three-dimensional morphology. Conversely, the principles of the ThinPrep method are dispersion, cell collection, and cell transfer.[14] First, the test filter rotates the sample vial and separates cells from mucus and other components. Then, cells are collected on the surface of the membrane by the vacuum within the test filter. After the test filter is inverted and pressed against the slide, positive air pressure causes the cells to adhere. Through these operations, cells are changed slightly into a two-dimensional form.

Because HGUC cells are three-dimensional on SurePath slides and slightly two-dimensional on ThinPrep slides, we inferred that the nuclear and cytoplasmic areas are significantly smaller on SurePath slides than on ThinPrep slides. Contrarily, the N:C ratio did not differ between the methods when all cells were analyzed. This is likely attributable

to the equal flatting ratio of the nucleus and cytoplasm in the ThinPrep method. Therefore, we considered the N:C ratio to be a good index of HGUC cells regardless of the LBC method used.

Conversely, the nuclear mean gray value, a surrogate for hyperchromasia, was significantly lower for the SurePath method than for the ThinPrep methods. In other words, the nucleus was darker on SurePath slides than on ThinPrep slides. This is probably the result of the three-dimensional structure of cells on SurePath slides. These results were similar to previous studies comparing qualitative cytomorphology using SurePath and ThinPrep.[9,11] Toyonaga et al.[11] reported that T24 cells (a cell line established from a patient with urinary bladder cancer) on ThinPrep slides exhibited cytoplasmic and nuclear flattening; meanwhile, severe cell shrinkage and hyperchromasia made nuclear details difficult to observe on SurePath slides in some cases. In addition, Michael et al.[9] described that nuclear shrinkage often decreases the N:C ratio using the SurePath method. However, based on our result using a digital image analysis system, there was no significant difference in the N:C ratio between SurePath and ThinPrep. It has been reported that the correlation between visual assessment and the true N:C ratio was sometimes insufficient in urinary cells.[15,16] Therefore, this discrepancy is likely attributable to the subjective visual assessment employed in the previous study.

Additionally, our study results illustrated that the mean nuclear roundness was significantly larger on ThinPrep slides than on SurePath slides; in other words, irregular nuclear borders were more prominent on SurePath slides than on ThinPrep slides. This result is likely because nuclear irregularity was weakened by the flattening of HGUC cells on ThinPrep slides.

In the analysis of the five cells with the largest N:C ratios, the average ratio exceeded 0.7 on both SurePath and ThinPrep slides, and HGUC cells with ratios exceeding 0.7 were present on almost all slides. In addition, the N:C ratio did not differ between the methods. Therefore, we considered an N:C ratio of 0.7 as a reasonable cutoff for detecting HGUC using the TPS. A previous study using the five cells HGUC cells with the largest N:C ratios reported mean ratios of 0.66 for ThinPrep slides and 0.64 for Cytospin slides, and these values did not significantly differ.[8] Consequently, although the statistical differences between the results of SurePath and Cytospin is unclear, the N:C ratio could be an index that is less affected by the processing method. Meanwhile, the nuclear gray value was significantly lower for SurePath than for ThinPrep in our study. The previous study reported that the nuclear gray value was significantly lower for ThinPrep than for Cytospin.[8] In other words, the degree of hyperchromasia differs depending on the processing method, and nuclear darkness is greatest in SurePath slides, less so in ThinPrep

slides, and least in Cytospin slides.

In summary, our current study revealed that the N:C ratio of HGUC cells is similar between the SurePath and ThinPrep methods. Additionally, HGUC cells with N:C ratios exceeding 0.7 were present on almost all slides in both methods. By contrast, hyperchromasia was significantly more severe on SurePath slides than on ThinPrep slides, and irregular nuclear borders were more prominent on SurePath slides than on ThinPrep slides. These data indicate the reasonableness of using the N:C ratio as the major criterion for the TPS and an N:C ratio cutoff of 0.7 for detecting HGUC cells. Further quantitative cytomorphologic studies of HGUC cells and normal and benign atypical cells on SurePath and ThinPrep slides are needed to evaluate each category described in the TPS.

REFERENCES

1. Charlton ME, Adamo MP, Sun L, Deorah S. Bladder cancer collaborative stage variables and their data quality, usage, and clinical implications: a review of SEER data, 2004-2010. *Cancer*. 2014;120 (0 23):3815-3825.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7-34.
3. Thiryayi SA, Rana DN. Urine cytopathology: challenges, pitfalls, and mimics. *Diagn Cytopathol*. 2012;40(11):1019-1034.
4. Straccia P, Bizzarro T, Fadda G, Pierconti F. Comparison between cytospin and liquid-based cytology in urine specimens classified according to the Paris System for Reporting Urinary Cytology. *Cancer Cytopathol*. 2016;124(7):519-523.
5. Rosenthal DL, Worjcik EM, Kurtycz DFI. *The Paris System for Reporting Urinary Cytology*. New York: Springer Science+Business Media; 2015.
6. Barkan GA, Wojcik EM, Nayar R, et al. The Paris System for Reporting Urinary Cytology: The quest to develop a standardized terminology. *Acta Cytol*. 2016;60(3):185-197.
7. VandenBussche CJ. A review of the Paris system for reporting urinary cytology. *Cytopathology*. 2016;27(3):153-156.

8. Richardson CJ, Pambuccian SE, Barkan GA. Split-sample comparison of urothelial cells in ThinPrep and cytospin preparations in urinary cytology: Do we need to adjust The Paris System for Reporting Urinary Cytology criteria? *Cancer Cytopathol*. 2020;128(2):119-125.
9. Michael CW, McConnel J, Pecott J, Afify AM, Al-Khafaji B. Comparison of ThinPrep and TriPath PREP liquid-based preparations in nongynecologic specimens: a pilot study. *Diagn Cytopathol*. 2001;25(3):177-184.
10. Park GS, Lee SH, Jung SL, Jung CK. Liquid-based cytology in the fine needle aspiration of parathyroid lesions: a comparison study with the conventional smear, ThinPrep, and SurePath. *Int J Clin Exp Pathol*. 2015;8(10):12160-12168.
11. Toyonaga Y, Yamazaki K, Koyama Y, Yamada M, Ishida Y. A modified direct-smear processing technique employing two-step centrifugation/fixation is useful for detecting high-grade urothelial carcinoma. *Acta Cytol*. 2017;61(6):447-454.
12. Hang JF, Charu V, Zhang ML, VandenBussche CJ. Digital image analysis supports a nuclear-to-cytoplasmic ratio cutoff value of 0.5 for atypical urothelial cells. *Cancer Cytopathol*. 2017;125(9):710-716.
13. Ohsaki H, Sofue T, Kawakami K, et al. WT1 immunoenzyme staining using SurePath (™) processed urine cytology helps to detect kidney disease. *Cytopathology*.

2016;27(1):43-49.

14. ThinPrep® 5000 Processor. Operator's Manual. Available from URL: https://www.hologic.com/sites/default/files/package-insert/MAN-02203-001_002_02.pdf. Accessed March 22, 2021.
15. Zhang ML, Guo AX, VandenBussche CJ. Morphologists overestimate the nuclear-to-cytoplasmic ratio. *Cancer Cytopathol*. 2016;124(9):669-677.
16. Layfield LJ, Esebua M, Frazier SR, et al. Accuracy and reproducibility of nuclear/cytoplasmic ratio assessments in urinary cytology specimens. *Diagn Cytopathol*. 2017;45(2):107-112.

Figure legends

Figure 1. Traced high-grade urothelial carcinoma cell nucleus on the ImageJ application window (Papanicolaou stain, original magnification ×100). (a) SurePath slide. (b) ThinPrep slide. Both images (a) and (b) are from the same patient.

Figure 2. (a) SurePath slide: the nuclear to cytoplasmic (N:C) ratio was 0.64; nuclear mean gray value was 107.3; and nuclear roundness was 0.97. (b) ThinPrep slide: the N:C ratio was 0.65; nuclear mean gray value was 141.4; and nuclear roundness was 0.96. Both images (a) and (b) are from the same patient. (Papanicolaou stain, original magnification

×100)

Figure 3. (a) SurePath slide: the nuclear to cytoplasmic (N:C) ratio was 0.78; nuclear mean gray value was 99.0; and nuclear roundness was 0.97 (b) ThinPrep slide: the N:C ratio was 0.75; nuclear mean gray value was 126.7; and nuclear roundness was 0.96. Both images (a) and (b) are from the same patient. (Papanicolaou stain, original magnification ×100)

Figure 4. (a) SurePath slide: the nuclear to cytoplasmic (N:C) ratio was 0.61; nuclear mean gray value was 69.3; and nuclear roundness was 0.90 (b) ThinPrep slide: the N:C ratio was 0.69; nuclear mean gray value was 85.8; and nuclear roundness was 0.98. Both images (a) and (b) are from the same patient. (Papanicolaou stain, original magnification ×100)

Author contributions

Methodology: Ohsaki H. **Formal analysis**: Okuda C., Nakamura A. and Ohsaki H. **Software**: Okuda C. and Nakamura A. **Visualization**: Okuda C. and Ohsaki H. **Resources**: Kyoutake A., Itoh T., and Ohsaki H. **Writing-original draft**: Okuda C. and Ohsaki H. **Writing-review and editing**: Kamoshida S. and Ohsaki H. **Supervision**:

Ohsaki H

Data availability statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

TABLE 1. Comparative of the quantitative cytomorphologic features of HGUC cells between SurePath and ThinPrep

Mean (Range)	SurePath	ThinPrep	*p* value
Nuclear area (μm^2)	168.0 (54.7–925.6)	185.2 (37.0–823.3)	<0.001
Cytoplasmic area (μm^2)	314.1 (97.5–1651.4)	347.5 (81.2–1151.2)	<0.001
N:C ratio	0.54 (0.32–0.88)	0.54 (0.31–0.81)	0.373
N:C ratio †	0.72 (0.54–0.88)	0.71 (0.55–0.81)	0.265
Nuclear mean gray value (Hyperchromasia)	93.9 (40.2–169.4)	110.8 (49.9–185.3)	<0.001
Nuclear mean gray value † (Hyperchromasia)	97.6 (48.8–169.4)	119.2 (72.3–165.5)	<0.001

Nuclear roundness (Irregular nuclear borders)	0.90 (0.62–0.96)	0.90 (0.56–0.96)	0.024

†: Five HGUC cells with the largest N:C ratios

HGUC: high-grade urothelial carcinoma

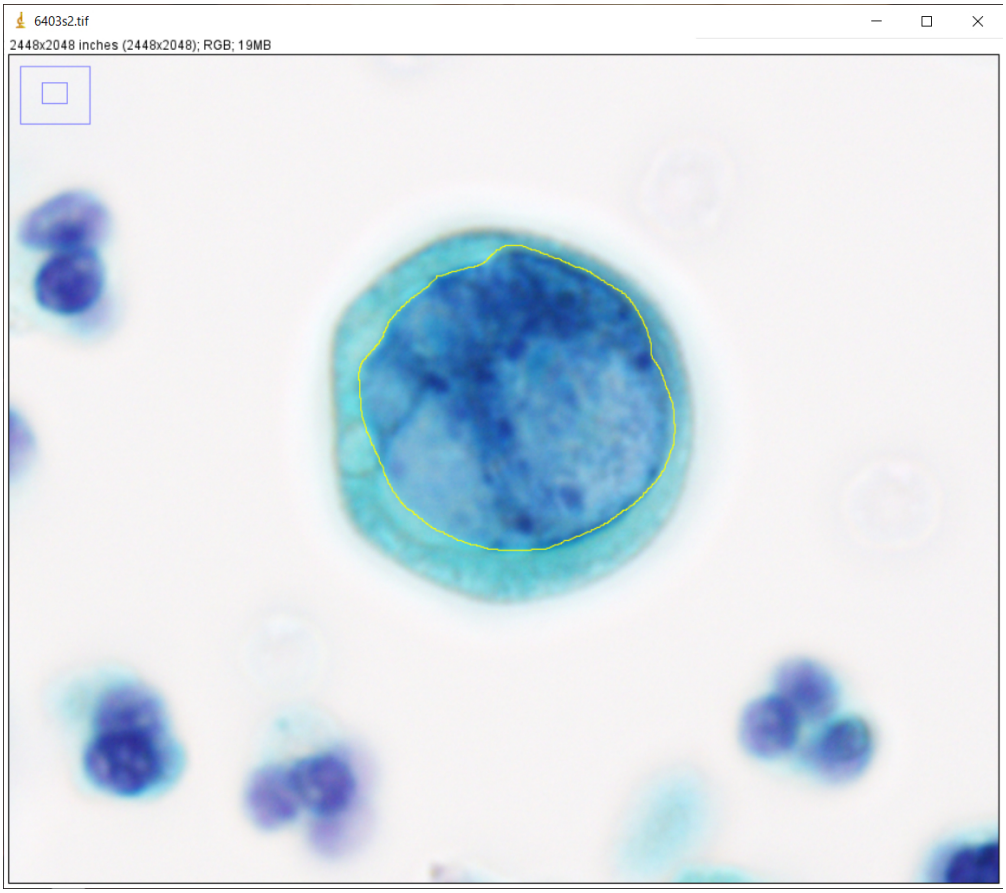

Fig 1. (a) SurePath slide

83x74mm (350 x 350 DPI)

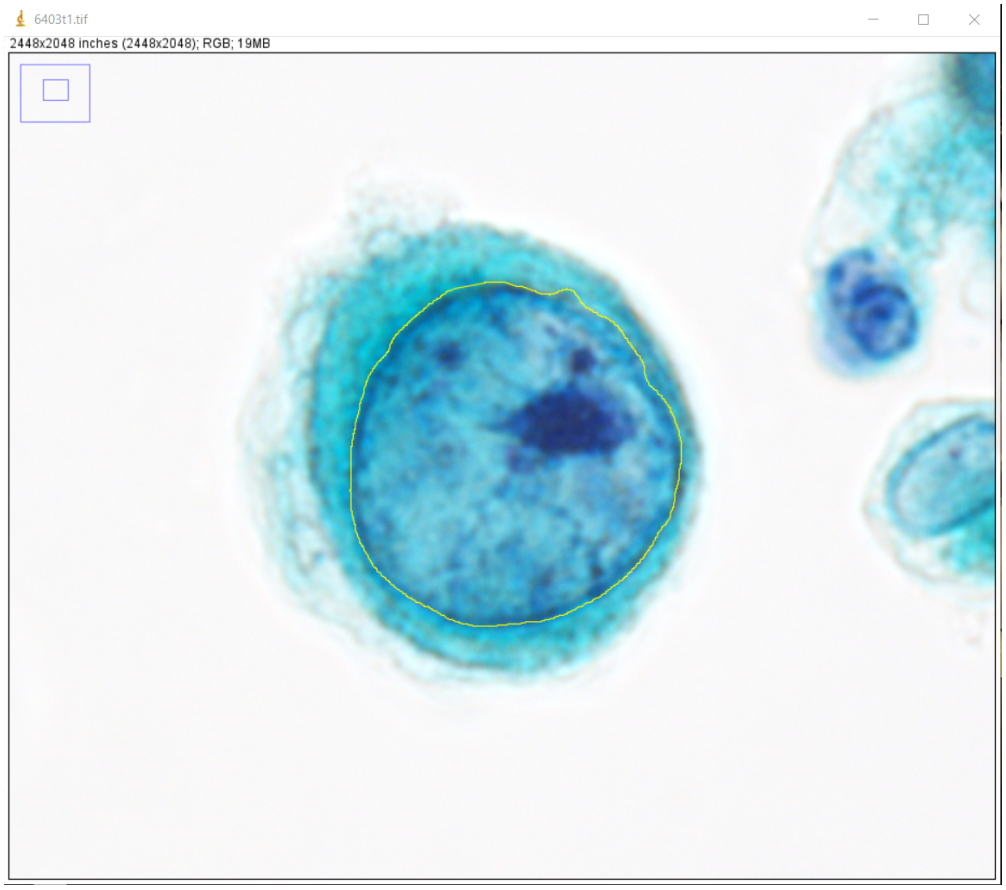

Fig 1. (b) ThinPrep slide

83x73mm (350 x 350 DPI)

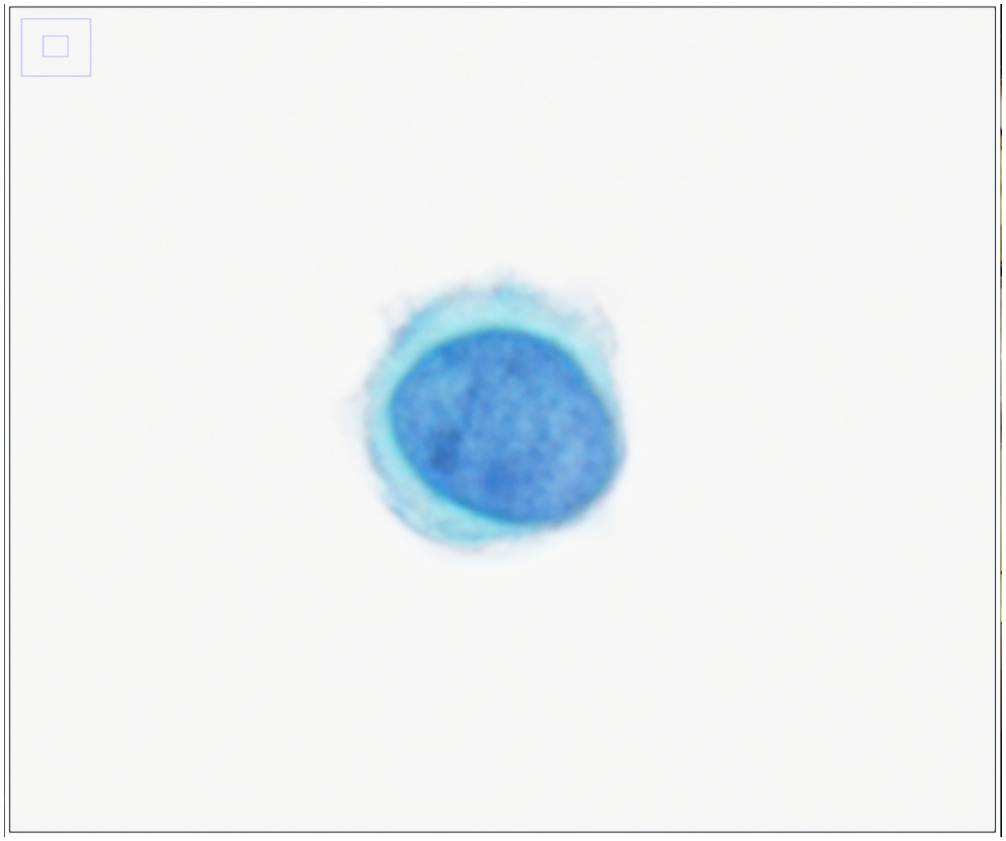

Fig 2 (a)

244x203mm (300 x 300 DPI)

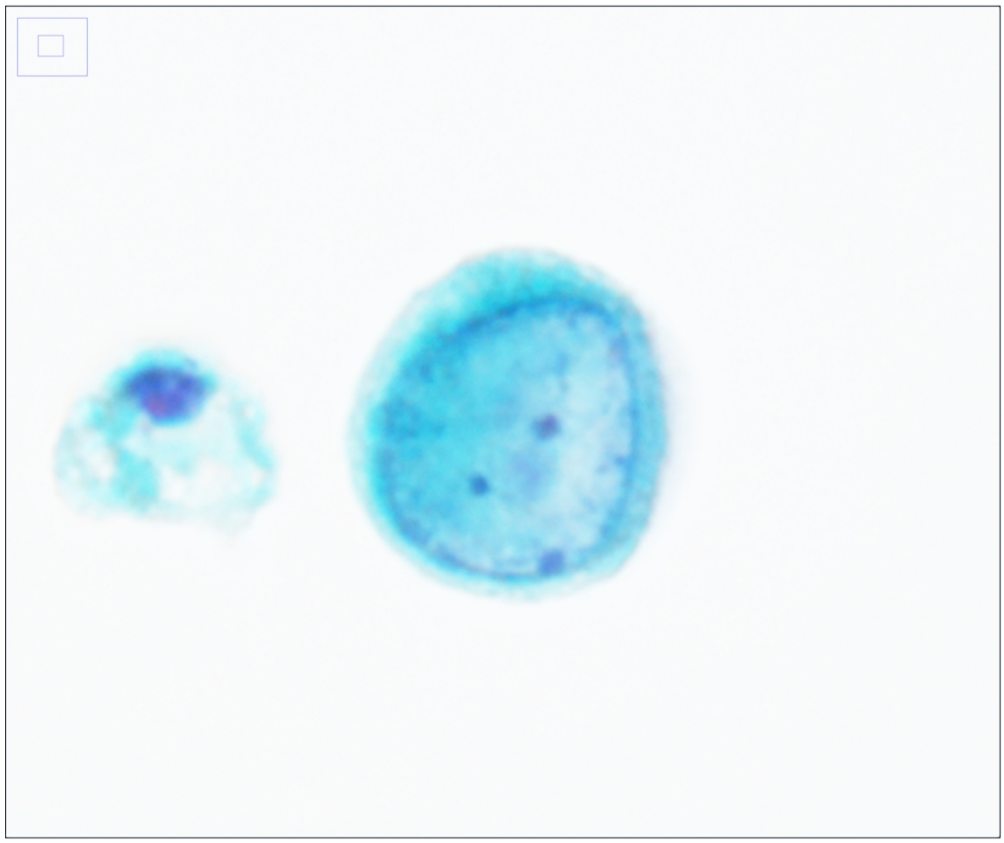

Fig 2 (b)

242x202mm (300 x 300 DPI)

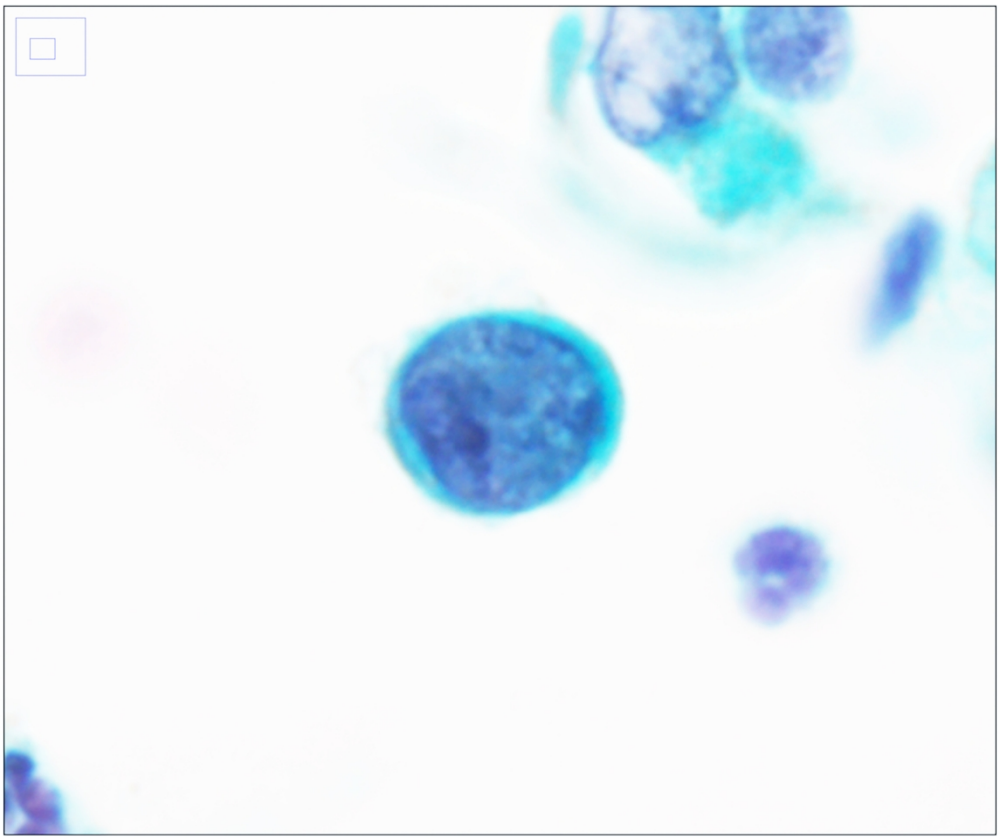

Fig 3 (a)

243x203mm (300 x 300 DPI)

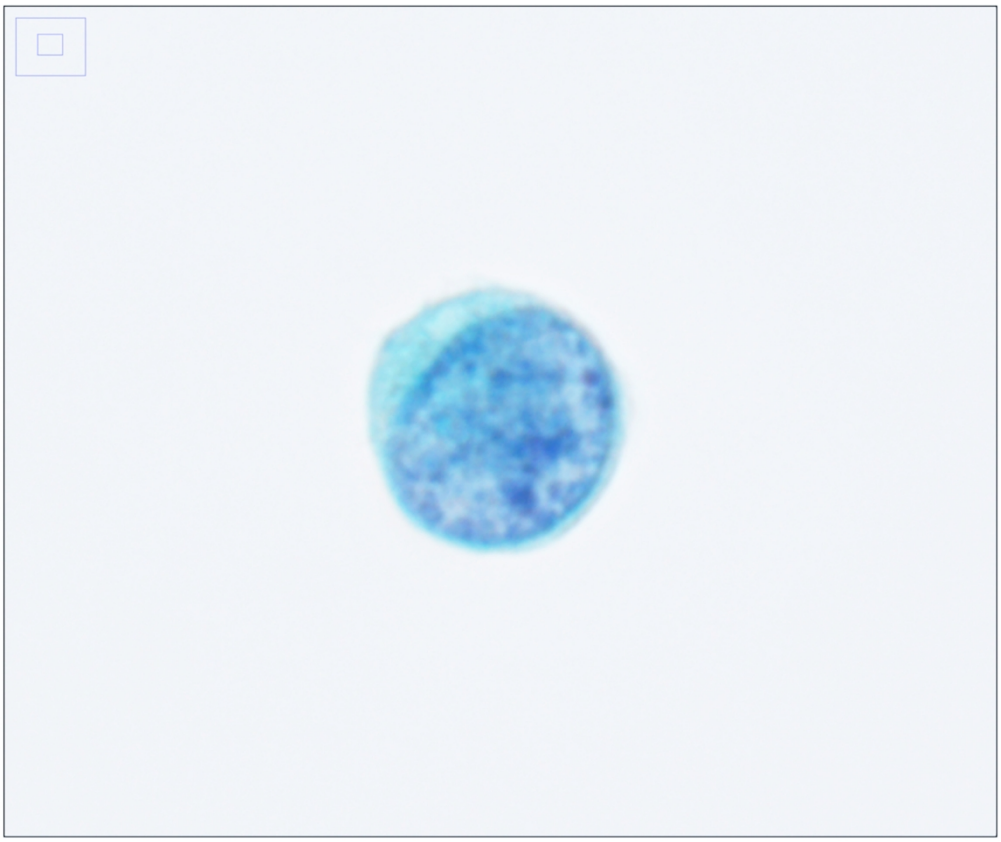

Fig 3 (b)

243x203mm (300 x 300 DPI)

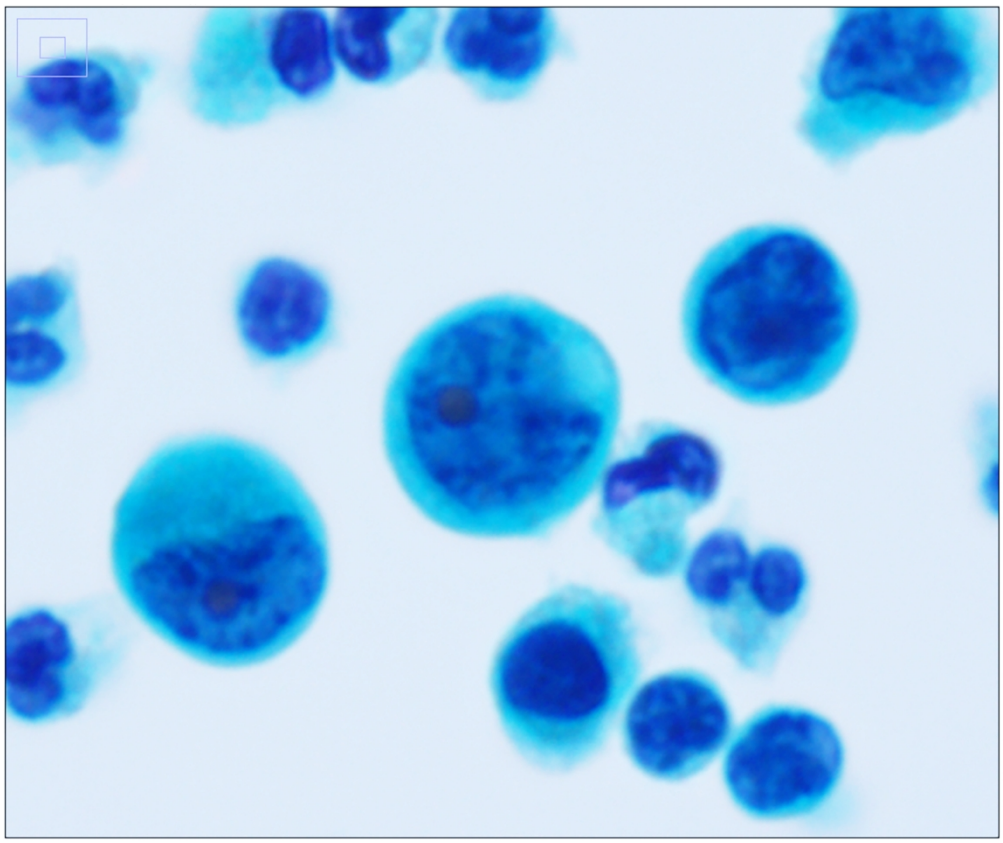

Fig 4 (a)

243x203mm (300 x 300 DPI)

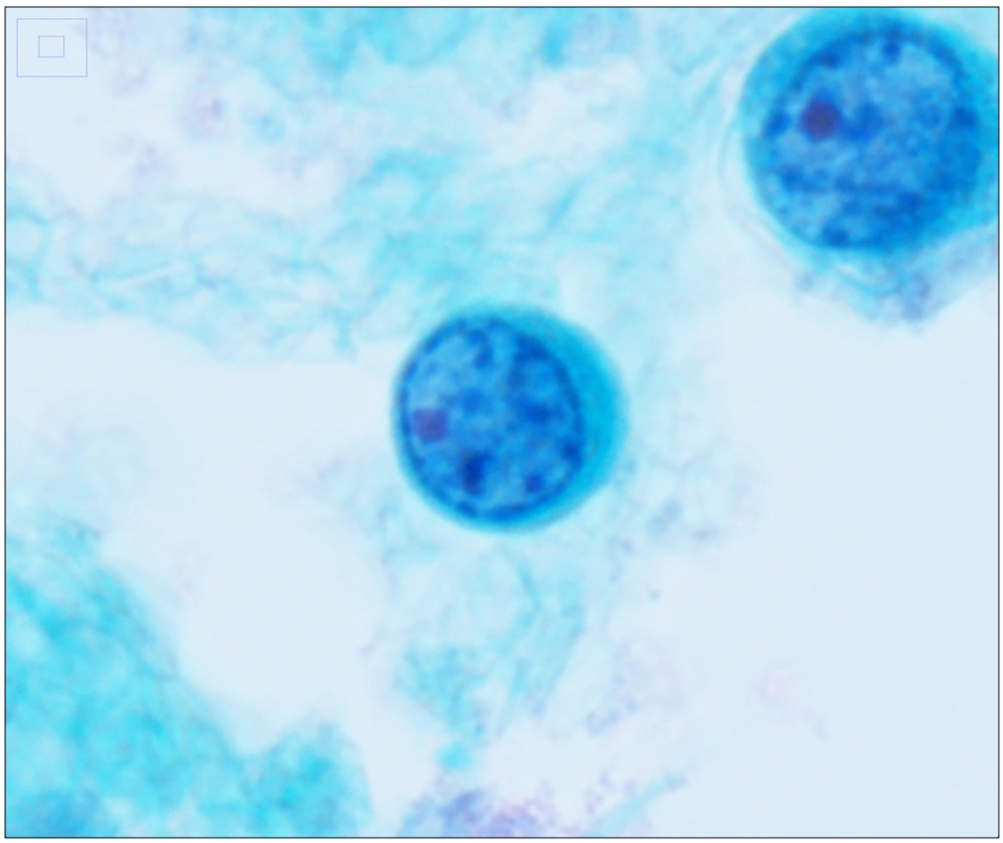

Fig 4 (b)

243x203mm (300 x 300 DPI)