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Title: Is there a role for the ThinPrep Imaging System in reporting anal cytology?

Short title: ThinPrep Imaging of anal cytology

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Abstract

Background: The ThinPrep Imaging System (TIS) is an accurate time-saving method of reading cervical ThinPrep slides in screening programs. As anal and cervical cytology are morphologically similar, TIS can potentially be used for anal cytology. We assessed the performance of TIS on anal ThinPrep slides from homosexual men in a natural history study of human papillomavirus-related anal abnormalities.

Methods: 419 anal cytology slides were processed by TIS and classified by a cytologist as either *No further review* (slide archived) or *Manual review* (slide requiring full manual screen). The results were compared with the original manual screening report for all slides and specifically for those screening episodes accompanied by a high-grade squamous intraepithelial lesion (HSIL) on concurrent biopsy.

Results: 176 of 419 (42.0%) slides were classified as *No further review*, with a trend of decreasing proportions as the degree of severity of the cytological abnormality increased. 13 (27.7%) slides with an original unsatisfactory report were classified as *No further review.* 82 (92.1%) of those with biopsy HSIL and cytological abnormality were classified for *Manual review*, including all 45 (100%) with cytological HSIL.

Conclusion: The cervical algorithm of TIS performed best on anal samples when HSIL was present both cytologically and histologically. The 27.7% unsatisfactory slides classified as *No further review* may indicate need for use of different criteria from cervical cytology. Because of the high prevalence of abnormalities, and hence the large proportion of slides needing manual review, the cytologist time-saving would compare unfavourably with use of TIS in cervical screening.

Key words: anal cytology, ThinPrep Imaging, high-grade squamous intraepithelial lesion

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Introduction

Cervical cytology-based screening programs have been responsible for marked reductions in cervical cancer incidence and mortality in many countries. As the incidence of anal carcinoma, most of which are also human papillomavirus (HPV)-associated tumours, rises in many parts of the world (1), cytological screening programs have been proposed for high-risk populations, in order to detect the anal cancer precursor lesion, high grade squamous intraepithelial lesion (HSIL).(2, 3) Anal programs may be based on the classic cervical screening triad of cytology, colposcopy and biopsy followed by treatment. (4)

Liquid-based cytology (LBC) has replaced conventional cytology in many countries with cervical cytological screening programs. In recent years, use of the ThinPrep Imaging System (TIS) ® (Bedford, MA, USA) has been shown to be an accurate and cost-effective method of reading cervical ThinPrep (TP) slides.(5-11) This system utilises computer imaging to present the cytologist with the fields most likely to contain abnormal cells. If no abnormal cells are seen in these fields, the slide can be immediately archived as negative, without manual screening of the whole slide. If abnormal cells are seen in these fields or the slide is suspected of being unsatisfactory, then full manual screening must be undertaken. The imager makes no assessment about the degree of abnormality present and final diagnostic decision-making remains with the cytologist and/or cytopathologist. In cervical screening programs in low-risk populations, over 90% of slides have no abnormality and so use of TIS reduces the tedium and screening time for cytologists and represents a significant labour cost saving over manual screening, without loss of accuracy.(5-8)

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Anal cytological appearances are morphologically similar to cervical cytological appearances.(4) In view of this similarity, we undertook to assess whether the TIS could be utilised for interpretation of anal cytological slides, using the cervical algorithm. A second aim was to assess whether the productivity gains observed in the use of TIS in cervical screening occurred in anal screening.

Materials and Methods

The Study of the Prevention of Anal Cancer (SPANC) is a longitudinal natural history study, exploring the epidemiology of anal HPV infection and related epithelial lesions among a community-recruited cohort of homosexual men in Sydney, Australia. Ethics approval for SPANC was granted by the Human Research Ethics Committee at the St Vincent's Hospital, 390 Victoria Street, Darlinghurst, NSW (HREC/09/SVH/168, Protocol Version 1.0 dated 20/04/2010, approved on April 22, 2010). All participants gave informed consent.

A detailed description of the design of SPANC has been published elsewhere.(12) In brief, the study recruited 617 homosexual men aged ≥ 35 years, both HIV positive (35.6%) and HIV negative, between September 2010 and August 2015 and is ongoing. Each participant has 5 clinic visits over a 3 year period. At each visit men undergo a digital anorectal exam and anal swab for cytology (ThinPrep) and HPV detection and genotyping. This is followed by high-resolution anoscopy (HRA) which directs the anoscopist to lesions suspected of being HPV-related for biopsy.

All cytological and histological specimens are referred to Gynaepath, a specialist ano-genital unit within Douglass Hanly Moir Pathology, a large private general pathology laboratory in Sydney. A total of five experienced cytologists have shared all cytological screening and

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three experienced anogenital pathologists have shared final reporting of all anal TP slides over the course of the study. Anal cytological reporting utilises the Bethesda system (TBS) 2001.(13) Anal histology specimens are reported by the same three pathologists using Lower Anogenital Squamous Terminology (LAST). (14)

For the purposes of this study we chose 450 TP slides (from 450 men), which had been previously reported by manual screening as part of SPANC. We excluded the first 6 months of SPANC to avoid the effects of the initial learning curve the cytologists experienced. Based on the proportion of cytology reports in each TBS category in SPANC at that time, we used stratified random sampling to select the slides: 11% Unsatisfactory, 38% Negative, 21% ASC-US/LSIL and 30% ASC-H/ HSIL.

All slides were scanned by the computer imaging process. As in cervical TIS use, a proportion of slides was 'rejected' for technical or unknown reasons. For the remainder of slides, the selected field co-ordinates were stored electronically on the server. The selected slides were retrieved and each was reviewed by one of three study cytologists at the 'review scope', with no knowledge of the original manual screening report. This review scope interfaces with the computer imager server and retrieves the stored co-ordinates. As is the usual process for cervical TP slides, the cytologist was presented with 22 fields of view (FOV) based on these co-ordinates. Twenty were chosen by the imager to represent any possible abnormality present on the slide (refer to Figures 1 and 2) and 2 were chosen to represent glandular groups. The cytologist viewed these 22 FOV and made a decision based on a suspicion of abnormality or of too few cells for assessment (defined as less than 2000 nucleated squamous cells by TBS 2001). If so, the slide was triaged as requiring full manual screening, termed here *Manual review*. However, we did not actually perform another full

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manual screen, as these slides had already been fully screened by a cytologist and checked by a pathologist. If no suspicion of abnormality or low cellularity arose, the slide was triaged as requiring *No further review*. We calculated the proportion of slides for *Manual review* and *No further review* for each TBS category, according to the original manual report. Figure 3 is a flow diagram summarising the study design.

For slides in which the concurrent histologic diagnosis was HSIL in at least one biopsy specimen, we compared the performance of manual reading and TIS triage.

Further, in order to make an approximate assessment of time-saving benefit of TIS, we measured the percentage of anal TP slides which would require full screening (*Manual review* percentage plus rejected percentage) and compared that with the percentage requiring full screening in cervical use of TIS.

Results

A sample of 450 TP slides had been selected. Nine (2.0%) were found to utilise glass slides not compatible with the imager and so these were excluded. 22 (5.0%) of the remaining 441 were rejected by the imager for technical reasons (eg coverslip problem) or for unknown reasons and so the entire review process took place for 419.

Table 1 presents the numbers and percentages of TP slides in the two categories: *Manual review* and *No further review*, for each TBS category, according to the original manual report. The percentage of slides in the *No further review* category, which in a screening program would be immediately archived as negative without full manual screening, was

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42.0% overall (95% CI 37.2-46.9) and ranged from 0% (97.5% one-sided CI 0-7.6) for HSIL – AIN 3 to 70.3% (95% CI 62.7-77.2) for Negative. There was a general trend of decreasing percentages of *No further review* as the degree of severity of the originally reported cytological abnormality increased.

Table 2 presents the original manual cytology report and the TIS category, for the 89 cases with concurrent biopsy confirmed HSIL and an abnormal original cytology report. When HSIL had been recognised on manual screening, zero such slides were archived as negative in the TIS arm. This rose to 2 of 22 when the original manual cytoprediction was ASC-H and 5 of 23 when the original manual cytoprediction was ASC-US or LSIL. We reviewed these 7 slides which were apparently erroneously archived as negative. In 2 slides, a few ASC-US cells actually were present in a single field of view chosen by the imager. In another 2 slides, ASC-US and LSIL cells were identified in fields not chosen by the imager. In one further slide, a single small cell with high nuclear:cytoplasmic ratio and a somewhat degenerate hyperchromatic nucleus was present in a field again not chosen by the imager. In the remaining 2 slides, no abnormal cells were seen in any of the 22 fields of view. Further, complete rescreening of these 2 slides revealed that the rescreeners (DE and JR) disagreed with the original manual report and considered the slides to be negative.

13 of 47 (27.7%) slides with an original unsatisfactory report were classified by TIS as *No further review*, so would have been called negative, rather than unsatisfactory (requiring a repeat sample).

Overall 243 slides (55.1%) were triaged for *Manual review* and 22 (5.0%) were rejected, giving a total of 265 slides (60.1%) requiring full manual screening.

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Discussion

In a natural history study of HPV-related anal abnormalities in homosexual men, we have demonstrated that TIS screening was able to mark for review all TP slides originally reported with cytological HSIL, and associated with histological HSIL. This indicates that the algorithm used for HSIL detection in TIS is accurate for anal cytology, due to its morphologic similarity to cervical cytology. TIS screening also marked for review the majority of TP slides originally reported with lesser cytological abnormalities and concurrent histological HSIL. That not all were marked for review is of concern because in an anal screening program, the finding of any cytological abnormality would typically lead to referral for HRA, because anal cytology is known to undercall the degree of abnormality present on histology.(4) Hence failure for TIS to identify all lesser abnormalities would result in some patients not being appropriately triaged to HRA. Our review of the 7 apparent 'misses' revealed both 'imager error' (2 ASC-US/LSIL slides and 1 ASC-H slide with only one abnormal cell) and 'human error' (4 examples of variation in morphological interpretation) were to blame. The latter is a reflection of the limits of cytological interpretation, especially in equivocal and low-grade categories and occurs whether the TIS is used or not.

A previous study comparing TIS and manual screening of anal TP slides showed similar sensitivities for detection of biopsy HSIL in the 2 arms.(15) To our knowledge, our study is the largest study assessing TIS screening of anal slides.

Overall, 27.7% of slides assessed as unsatisfactory due to low cellularity by manual screen were deemed *No further review* by TIS. As TIS makes no determination regarding cellularity, the cytologist is required to assess this as the slide moves from field to field. In contrast to

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cervical Paps, anal cytology samples generally have many anucleate squamous cells in the background. These are ignored in assessment of cellularity, which depends only on the number of nucleate squamous cells present. The results reported here are most likely due to cytologists trained on cervical TP slides not taking this into account and mistakenly deciding that a sample with few nucleated squamous cells was actually satisfactory. In a screening program, these results would translate as approximately one quarter of the men who should be recalled for a repeat anal cytology test due to low cellularity, instead being reassured that their test was negative. This level of false reassurance would be unacceptable in a screening program and cytologists would need to be educated accordingly.

The rejected rate of 5.0% is comparable to the proportion of rejected slides (3.7%) in our cervical TIS study. (7) The *Manual review* rate plus rejected rate resulted in over 60% of slides requiring full manual review. This compares very unfavourably with approximately 16% for cervical TP slides read with TIS (unpublished data). In cervical screening, TIS produces a time-saving benefit related to the *No further review* rate, derived from effectively doubling screening productivity by cytologists, as reported in two Australian studies. (6, 7) Because of the high rate of anal cytological abnormality (51%) producing a nearly four-fold increase in the number of anal TP slides for full screening compared with cervical cytology (with an abnormality rate of <5%), there is unlikely to be a large time-saving benefit in TIS use in anal screening.

In summary, our results indicate that the algorithm used by TIS for cervical TP reading will enable detection of biopsy-confirmed anal HSIL. The efficacy of TIS is higher when the cytological abnormality is more severe. Education of cytologists would be needed to reduce the high rate of poorly cellular slides deemed negative by TIS. Finally, with the proportion of

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slides requiring full review being much higher than for cervical specimens (due to a higher prevalence of disease), TIS is unlikely to produce as great benefit in terms of cytologist time saved. These data may be useful if and when decisions are made about development of an anal screening program and the cost-effectiveness thereof.

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Figure Legends:

Figure 1: Anal ThinPrep slide with field of view electronically selected and marked by ThinPrep Imaging System. Low power x 4

Figure 2: High power of the field of view in Figure 1 showing squamous cells predictive of high-grade squamous intraepithelial lesion. High power x 40

Figure 3: Summary of study design

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Table 1: Numbers (and percentages) of ThinPrep slides classified by original (manual screening) cytologic reporting category and ThinPrep Imaging System category

	ThinPrep Imaging System Category				
Manual Screening Report	*Manual review*	95% CI	*No further review*	95%CI	Total
All	243 (58.0%)	53.1-62.8	176 (42.0%)	37.2-46.9	**419**
Unsatisfactory	34 (72.3%)	57.4-84.4	13 (27.7%)	15.6-42.6	47
Negative	49 (29.7%)	22.8-37.3	116 (70.3%)	62.7-77.2	165
ASC-US	26 (46.4%)	33.0-60.3	30 (53.6%)	39.7-67.0	56
LSIL	28 (82.4%)	65.5-93.2	6 (17.6%)	6.8-34.5	34
ASC-H	48 (82.8%)	70.6-91.4	10 (17.2%)	8.6-29.4	58
HSIL – AIN 2	11 (91.7%)	61.5-99.8	1 (8.3%)	0.2-38.5	12
HSIL – AIN 3	47 (100.0%)	92.5-100*	0 (0%)	0-7.6*	47
Total	243	-	176	-	**419**

*97.5% one-sided confidence interval.
ASC-US = Atypical squamous cells of undetermined significance
LSIL = Low-grade squamous intraepithelial lesion
ASC-H = Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion
HSIL = High-grade squamous intraepithelial lesion
AIN = Anal intraepithelial neoplasia

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Table 2: Numbers (and percentages) of ThinPrep slides classified by original (manual screening) abnormal reporting category and ThinPrep Imaging System category for cases with concurrent biopsy confirmed HSIL

	ThinPrep Imaging System Category				
Manual Screening Report	*Manual review*	95% CI	*No further review*	95% CI	**Total**
ASC-US/LSIL	18 (78.3%)	56.3-92.5	5 (21.7%)	7.5-43.7	23
ASC-H	19 (90.5%)	69.6-98.8	2 (9.5%)	1.2-30.4	21
HSIL	45 (100%)	92.1-100.0*	0 (0%)	0-7.9*	45
Total	82 (92.1%)		7 (7.9%)		**89**

*97.5% one-sided confidence interval.

ASC-US = Atypical squamous cells of undetermined significance
LSIL = Low-grade squamous intraepithelial lesion
ASC-H = Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion
HSIL = High-grade squamous intraepithelial lesion

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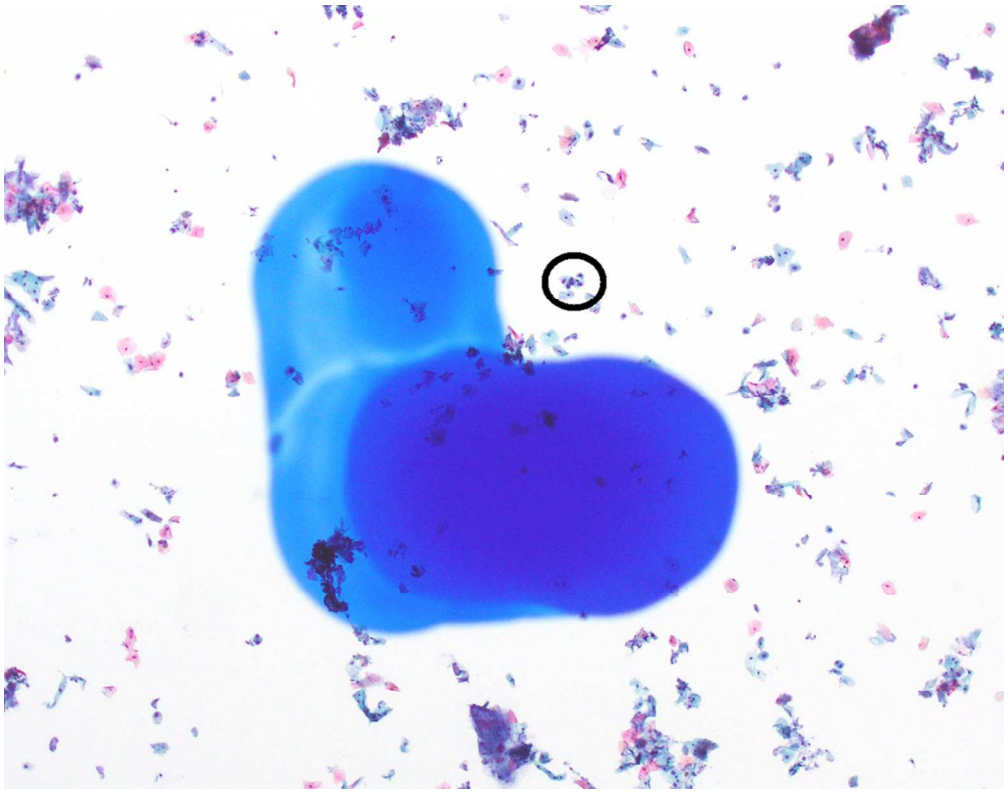


Figure 1: Anal ThinPrep slide with field of view electronically selected and marked by ThinPrep Imaging System. Low power x 4
103x81mm (300 x 300 DPI)

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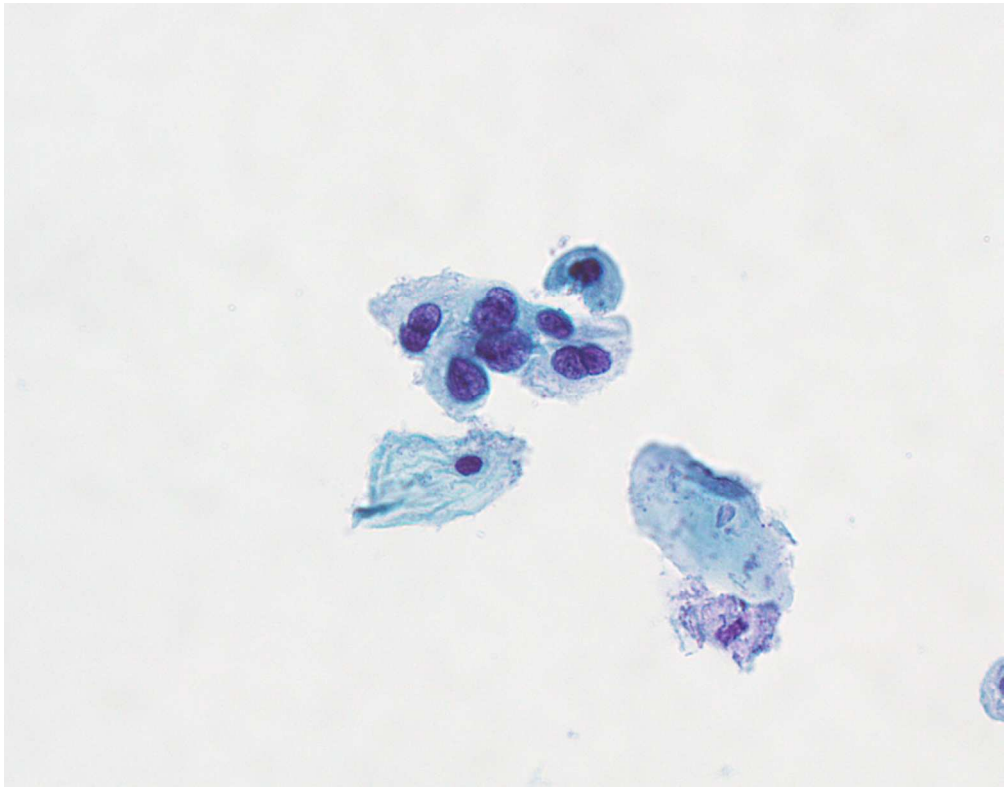


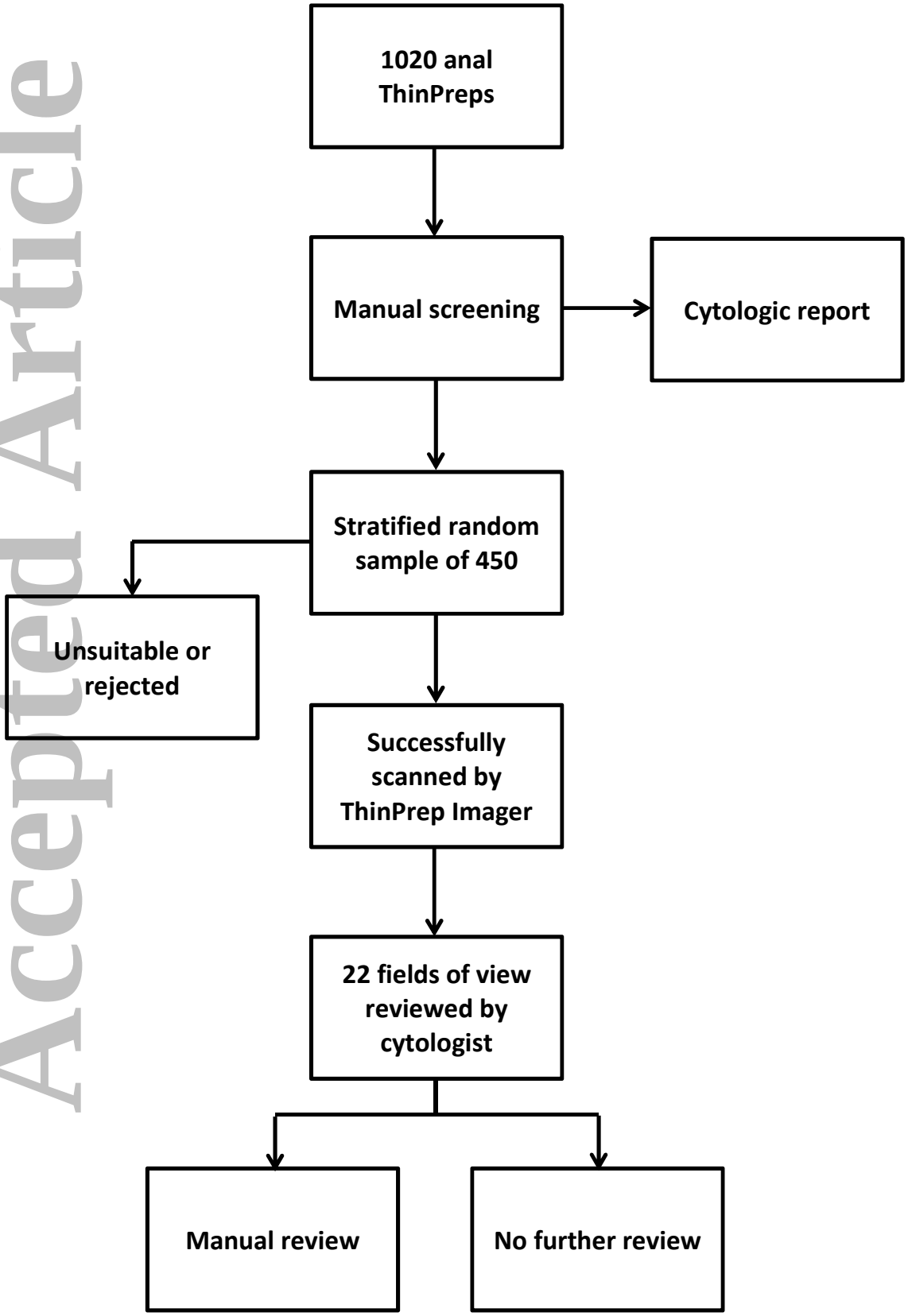
Figure 2: High power of the field of view in Figure 1 showing squamous cells predictive of high-grade squamous intraepithelial lesion. High power x 40
103x81mm (300 x 300 DPI)

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Figure 3



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