

difference in the performance of liquid based cytology compared with conventional cytology according to experience with liquid based cytology, nor with increasing experience in the study. Also, point estimates changed little when we restricted the analysis to centres with experience of ThinPrep, or to the second half of enrolment in each centre.

On the basis of this analysis, the main advantage of moving to liquid based cytology is a reduction in the rate of unsatisfactory slides. Other advantages are the shorter time needed for interpretation^{4,5} and using the same sample for testing for human papillomavirus and for other molecular tests.

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Ethical approval: This study was approved by the local research ethics committees of the participating centres.

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Accuracy of reading liquid based cytology slides using the ThinPrep Imager compared with conventional cytology: prospective study

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ABSTRACT

Objective To compare the accuracy of liquid based cytology using the computerised ThinPrep Imager with that of manually read conventional cytology.

Design Prospective study.

Setting Pathology laboratory in Sydney, Australia.

Participants 55 164 split sample pairs (liquid based sample collected after conventional sample from one collection) from consecutive samples of women choosing both types of cytology and whose specimens were examined between August 2004 and June 2005.

Main outcome measures Primary outcome was accuracy of slides for detecting squamous lesions. Secondary outcomes were rate of unsatisfactory slides, distribution of squamous cytological classifications, and accuracy of detecting glandular lesions.

Results Fewer unsatisfactory slides were found for imager read cytology than for conventional cytology (1.8% v 3.1%; P<0.001). More slides were classified as abnormal by imager read cytology (7.4% v 6.0% overall and 2.8% v 2.2% for cervical intraepithelial neoplasia of grade 1 or higher). Among 550 patients in whom imager read cytology was cervical intraepithelial neoplasia grade 1 or higher and conventional cytology was less

severe than grade 1, 133 of 380 biopsy samples taken were high grade histology. Among 294 patients in whom imager read cytology was less severe than cervical intraepithelial neoplasia grade 1 and conventional cytology was grade 1 or higher, 62 of 210 biopsy samples taken were high grade histology. Imager read cytology therefore detected 71 more cases of high grade histology than did conventional cytology, resulting from 170 more biopsies. Similar results were found when one pathologist reread the slides, masked to cytology results.

Conclusion The ThinPrep Imager detects 1.29 more cases of histological high grade squamous disease per 1000 women screened than conventional cytology, with cervical intraepithelial neoplasia grade 1 as the threshold for referral to colposcopy. More imager read slides than conventional slides were satisfactory for examination and more contained low grade cytological abnormalities.

INTRODUCTION

Liquid based cytology for cervical smears is replacing conventional cytology in many countries yet evidence is insufficient to confirm that it is more accurate than conventional cytology.¹

High grade histology results for discordant cytology

	Reference standard								
	PTR report			Rereading result			More severe of PTR report and rereading result		
	No of slides	% of discordant cytology	% of histology	No of slides	% of discordant cytology	% of histology	No of slides	% of discordant cytology	% of histology
Imager read slide positive, conventional slide negative:									
Discordant cytology*	550	—	—	550	—	—	550	—	—
Histology obtained	380	69.1	—	280	50.9	—	380	69.1	—
High grade histology†	133	24.2	35.0	153	27.8	54.6	196	35.6	51.6
Imager read slide negative, conventional slide positive:									
Discordant cytology*	294	—	—	294	—	—	294	—	—
Histology obtained	210	71.4	—	154	52.4	—	210	71.4	—
High grade histology†	62	21.1	29.5	68	23.1	44.2	90	30.6	42.9

PTR=New South Wales Pap test register.

*Cervical intraepithelial neoplasia grade 1 cytology threshold.

†Cervical intraepithelial neoplasia grade 2 histology threshold.

Conventional cytology involves the transference of cervical material from a collection instrument to a glass slide whereas liquid based cytology involves rinsing the instrument in liquid to produce a suspension, which is then processed in a laboratory. The ThinPrep Imager (Cytec, Marlborough, MA) system is a new technology applied to liquid based cytology. The imager identifies 22 fields of interest most likely to contain abnormal cells.

As liquid based cytology has not been approved for government funding in Australia² it is sometimes carried out as an additional test within a split sample specimen—the conventional slide is made first and a liquid based slide is then made from residual material on the collection instrument. The Douglass Hanly Moir pathology laboratory offers manual reading of split sample liquid based cytology specimens. It recently introduced the ThinPrep Imager system.

We compared the accuracy of liquid based cytology using the imager with that of conventional cytology in detecting squamous lesions. Secondary outcomes were the rate of unsatisfactory slides, the distribution of squamous cytological classifications, and accuracy in detecting glandular lesions.

METHODS

We cross classified independently read imager read and conventional squamous cytology and measured the proportion with cervical intraepithelial neoplasia grade 2 or higher. This was carried out among slides positive with the imager and negative with conventional cytology and negative with the imager and positive with conventional cytology. We considered the results to be concordant if they were identical between two slides or if the recommendations for clinical management³ were the same for both results.

We compared results using three specific reference standards: histology reports from the New South

Wales Pap test register, masked rereading of histology results by a pathologist (AF), and the more severe results of the two.

Inclusion criteria were consecutive cervical specimens taken from women of any age who chose to have a liquid based cytology sample taken in addition to a conventional cytology sample, and whose specimens were examined between August 2004 and June 2005.

Liquid based cytology slides were read by the ThinPrep Imager. The 22 fields of interest were examined manually by a cytologist to locate possible abnormalities. Conventional cytology slides were read manually by a cytologist. The same evaluation process was then followed independently for both slides. Those considered normal were reported as such. If a slide was unsatisfactory or abnormal it was rescreened by a more experienced cytologist. If the cytologists agreed that a slide was unsatisfactory or normal, it was reported as such. If the cytologists agreed that a slide was satisfactory for examination but could not reach consensus about normality, the slide was reviewed by a pathologist. Thus two reports were made for each pair of slides. Cytologists and pathologists were masked to the cytology report on the other slide of the pair.

The final report issued to the referring clinician showed the worse abnormality for each slide. If both slides contained any abnormality the report stated that both slides showed the final result. In these cases decisions to colposcope and biopsy were made using a final cytology result that did not identify which technology yielded the more severe abnormality and were, therefore, unbiased. If one slide appeared normal and the other showed an abnormality, the identity of the technology that had detected the abnormality was stated.

We cross tabulated the cytology pairs according to whether they were satisfactory or unsatisfactory. Pairs with satisfactory slides were cross tabulated according

to their squamous cytology results. We used the threshold of cervical intraepithelial neoplasia grade 1 to determine positive and negative cytology for analyses of the accuracy of squamous cytology, because guidelines current during the study recommended referral of women with reports of cervical intraepithelial neoplasia grade 1 or higher for colposcopy.

For discordant pairs we sought the histology reports from the Pap test register until six months after the date of cytology. If more than one specimen was taken, we used the most severe result. We then cross tabulated and examined the results from the register with those of the masked rereadings. We categorised cervical intraepithelial neoplasia grade 1 or less severe histology as reference standard negative and grades 2 or 3 or carcinoma histology as reference standard positive.

For discordant results we calculated the numbers of reference standard positive and negative cases for both types of slide using cervical intraepithelial neoplasia grade 1 as the test threshold. We used the same methods for the additional analyses, using rereading of the slides as a reference standard and the more severe of the results from the register and results of masked histology rereading.

Satisfactory slide pairs, containing endocervical components, were cross tabulated according to classification of glandular cells. We examined the accuracy of abnormal glandular cytology at two thresholds: glandular atypia and glandular inconclusive. We considered adenocarcinoma in situ or adenocarcinoma as reference standard positive and negative glandular histology as reference standard negative.

Statistical analyses

We used McNemar's test to determine whether the proportions of unsatisfactory slides differed between the technologies. This method was also used to compare the accuracy of the two types of slide (cervical intraepithelial neoplasia grade 1 as test threshold) for the detection of high grade histology.

We compared the distribution of cytology classifications for both types of slide using a logistic generalised estimating equation model to take account of the pairing of results for each slide. The outcome variable was slide type, with test result fitted as a categorical explanatory variable.

RESULTS

Overall, 55 164 split sample pairs (liquid based slide obtained after conventional cytology slide from one collection) were evaluated.

Fewer slides were found to be unsatisfactory when read by the ThinPrep Imager: 982 (1.8%) *v* 1704 (3.1%); $P < 0.001$. Both imager read and conventional slides were satisfactory in 52 665 pairs (see [bmj.com](#)).

The distribution across squamous classifications differed between imager read and conventional cytology ($P < 0.001$, see [bmj.com](#)). The imager labelled more slides as containing low grade abnormalities than did conventional cytology. The imager was more likely to classify slides as atypia (odds ratio 1.08, $P = 0.018$),

atypia with signs of human papillomavirus infection (1.65, $P < 0.001$), and cervical intraepithelial neoplasia grades 1 (1.47, $P < 0.001$), 2 (1.45, $P < 0.001$), and 3 (1.15, $P = 0.019$), whereas the imager was less likely to classify slides as inconclusive, high grade histology to be excluded (0.78, $P = 0.0028$). The imager detected significantly more high grade histology, irrespective of reference standard used.

Of 550 pairs in which the imager read slide was reported as cervical intraepithelial neoplasia grade 1 or higher (positive cytology) and the conventional slide was reported as less than grade 1 (negative cytology; see [bmj.com](#)), 380 biopsy samples (69.1%) were available from the Pap test register (see [bmj.com](#)). Of 294 cytology pairs in which the conventional slide was reported as cervical intraepithelial neoplasia grade 1 or higher and the liquid based slide was less than grade 1, 210 biopsies (71.4%) were available. These percentages did not differ ($P = 0.48$). Histology rates were similar for more detailed cytology cross tabulation, for discordant pairs where at least one result was cervical intraepithelial neoplasia grade 1 or higher, and for the subset in which one slide of each pair was normal and the other was cervical intraepithelial neoplasia grade 1 or higher.

Among the 380 results from the Pap test register that were positive by imager read cytology and negative by conventional cytology, 133 (24%) were cervical intraepithelial neoplasia grade 2 or higher (table). Among the 210 Pap test register results that were negative by imager read cytology and positive by conventional cytology, 62 cases (21%) were high grade disease (see [bmj.com](#)). The imager therefore detected 71 more cases of histological high grade disease and required 170 more biopsy specimens to detect these cases. This increased detection was significant ($P < 0.001$).

Of 550 pairs in which only the imager read slide was reported as cervical intraepithelial neoplasia grade 1 or higher, 280 histology slides (50.9%) were reread masked to cytology results (table). Of 294 pairs in which only the conventional cytology slide was reported as cervical intraepithelial neoplasia grade 1 or higher, 154 histology slides (52.4%) were reread. These percentages did not differ ($P = 0.68$). Among the 280 histology slides reread for cytology pairs that were positive by imager read cytology and negative by conventional cytology, 153 (27.8%) were cervical intraepithelial neoplasia grade 2 or higher (see [bmj.com](#)). Among the 154 histology slides reread for cytology pairs that were negative by imager read cytology and positive by conventional cytology, 68 (23.1%) were cervical intraepithelial neoplasia grade 2 or higher. Therefore the imager detected 85 more cases of high grade disease than conventional cytology and required 126 more biopsies to detect these cases (table). This increased detection was significant ($P < 0.001$).

The register record and result of masked rereading of histology agreed in 81% of cases ($\kappa = 0.62$). To reduce misclassification the two histological assessments were combined, using the more severe of the two results as the reference standard. Imager read cytology detected

WHAT IS ALREADY KNOWN ON THIS TOPIC

The accuracy of liquid based cytology read using an imager compared with manually read conventional cytology is not known

WHAT THIS STUDY ADDS

Liquid based slides read using the ThinPrep Imager detected 1.3 more cases of high grade disease per 1000 women screened than did manually read conventional slides

3.1 more biopsies per 1000 women screened were required to detect these cases

106 more cases of high grade disease than conventional cytology and required 170 more biopsies to detect these cases (see [bmj.com](#)). This increased detection was significant ($P < 0.001$).

Glandular lesions

Of 35 599 pairs in which both slides were satisfactory and contained endocervical components, only 63 pairs contained at least one abnormal slide (see [bmj.com](#)). The imager labelled 23 slides and conventional cytology labelled 52 slides as glandular abnormalities. Of the 63 pairs containing abnormalities, 26 had results in the register. Seventeen of 24 abnormal pairs with at least one slide reported as either inconclusive, high grade histology to be excluded or high grade glandular cytology had register results available, whereas 10 of 12 pairs with at least one slide reported as high grade had register results available. Six of the 26 histology reports were adenocarcinoma in situ or adenocarcinoma.

Cross tabulation of the 26 verified cases by cytology and histology results showed that all cases of adenocarcinoma in situ or adenocarcinoma were detected by both technologies at the cytology threshold of atypia (see [bmj.com](#)). One case of high grade histology was detected only by conventional cytology at the cytology threshold of inconclusive, high grade histology to be excluded (see [bmj.com](#)). This case would, however, have been referred for colposcopy on the basis of the diagnoses of cervical intraepithelial neoplasia grade 3 for both technologies.

DISCUSSION

Liquid based cytology slides obtained under routine clinical practice and read using the ThinPrep Imager detected significantly more histological high grade disease than did manually read conventional cytology slides.

Although both technologies resulted in similar proportions of cases requiring biopsies because of discordant cytology (69% liquid based, 71% conventional), Imager read cytology resulted in a significantly greater yield of high grade histology from these biopsies. Among discordant cytology of cervical intraepithelial neoplasia grade 1 or higher, significantly more high grade histology (\geq grade 2) was detected by imager read cytology using three reference standards: histology results from the New South Wales Pap test register, masked rereading of histology, and the more severe of these two results.

The ThinPrep Imager detected 1.29 more cases of histological high grade disease per 1000 women screened than conventional cytology. For each additional 100 cases of high grade disease detected by the imager 240 biopsies would be required. When masked rereading of histology was used as the reference standard, 148 biopsies would be required for each additional 100 cases of high grade disease detected by the imager.

This study provides a valid comparison of the accuracy of the technologies for several reasons. Firstly, we avoided reporting bias by using masked reading of slides blinded to cytology results. Secondly, when both slides showed abnormal results, clinical decisions about whether to undertake colposcopy and biopsy were unbiased because clinicians were masked to knowledge of the technologies. When one slide showed normal results and one abnormal results, clinicians were potentially aware of which technology detected the abnormality. We found no evidence that the proportions biopsied differed for results positive by both technologies in these cases. Thirdly, our finding of the improved accuracy of the imager was consistent across three reference standards. Fourthly, we used split sample specimens thereby maximising the cellular content and potentially improving the accuracy of conventional cytology.

In Australia 7.7 cases of histologically confirmed high grade disease per 1000 women screened are detected each year through a biennial screening programme using conventional histology.⁴ On the basis of the results of this study introduction of the ThinPrep Imager would increase detection by 1.3 cases per 1000, to 9.0 cases per 1000 women screened, and would require 3.1 more biopsies per 1000 women screened to detect these cases.

Fewer liquid based slides have been classified as unsatisfactory when read by the Imager than when read manually.⁵ We found that the percentage of unsatisfactory imager read slides was 1.78% compared with 3.09% for conventional slides. Therefore fewer women might be recalled for repeat tests than is currently the case if the Imager was introduced into population screening programmes.

In this study more squamous cytological abnormalities were found by imager reading than by conventional cytology, except for those classified in the category inconclusive, high grade histology to be excluded. This reduction in the number of inconclusive slides read by the imager may be explained by the improved preservation and concentration of cells. This finding should result in a reduction in the number of women requiring colposcopy.

Apart from cytology reports of cervical intraepithelial neoplasia grade 1 or higher, which we examined against a histological reference standard, less severe cytological abnormalities were also reported in greater numbers by the imager than by conventional cytology. The significant increase we found in cases of atypia, with or without signs of human papillomavirus infection, detected by the imager might reflect improved detection of abnormalities associated with oncogenic human papillomavirus types.⁶ The imager

classified 8.6 more slides in these categories per 1000 women screened than did conventional cytology. However, it is estimated that about half of these abnormalities will regress to normal.⁷ The increased detection of low grade cytological lesions by the imager might result in higher rates of further testing. On the other hand, together with our finding of improved detection of histological cervical intraepithelial neoplasia grade 2, it does raise the possibility that the increased detection of squamous abnormality by the Imager might allow longer screening intervals.

Although more glandular abnormalities were reported with conventional cytology than with the Imager, the two were equivalent in detecting reference standard positive cases at a referral threshold of atypia. The decreased detection of cytological glandular lesions by the ThinPrep Imager in our study was not at the cost of reduced detection of histological glandular disease, although this result is limited by the small number of cases.

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Commentary: Liquid automation refreshes Dr Papanicolaou

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That the Papanicolaou test has been modified so little during the greater part of its existence is surely a tribute to the insight and inventiveness of the physician scientist from whom it takes its name. When change began to be mooted in the 1980s it was because new levels of computing power were making it realistic to think of automating the microscopic examination of cells from the cervix. But to do so reliably depended on first improving the quality of the smears. This prompted the development of liquid based cytology—and with it a need for realistic comparative assessments: of the performance of the new technique as against conventional methods,¹ and of automated as against manual microscopy for examining cervical cell preparations.²

Various liquid based cytology systems are now commercially available. In methods and equipment their details differ from one manufacturer to another, but all dispense with a core feature of the conventional cervical smear test. Instead of smearing the cervical material on to a microscope slide, the tester rinses the brush-like sample collection device in a small pot of preservative fluid. At the cytology lab the floating cells are dispersed and variously

centrifuged or filtered to remove blood, mucus, and other debris, and then allowed to form a monolayer on a microscope slide.

In principle this approach confers several advantages. Virtually all the cells collected from the cervix should be present in the suspension, allowing the final sample to be more representative of the original population. The cells themselves, besides being more evenly distributed across the slide, should also be better preserved. Consequently, the proportion of specimens rejected as unsuitable for examination should fall, allowing the productivity of cytology labs to rise. Laboratory staff taking part in a UK Department of Health evaluation study generally approved the system.³

Moving on to automation, here too one of the goals is an increase in productivity—coupled, it has been hoped, with improved accuracy. The ThinPrep imaging system used by Davey et al² is intended not to replace human judgment, but to facilitate it by drawing the attention of the microscopist to the cells of most relevance.⁴ It uses a stain that gives an appearance similar to that used in the conventional smear test, but in which the intensity of colouration is closely