IN PRACTICE

Blind sampling is superior to anoscope guided sampling for screening for anal intraepithelial neoplasia

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Objective: Analyt cytology smears are either collected “blind” (swab inserted 4 cm into anal canal and rotated) or guided through an anoscope (transformation zone visualised and then sampled). We compared these smear techniques with respect to sample quality and patient acceptability.

Methods: Using a paired, random sequence clinical trial, 151 homosexual men (n = 95 HIV positive) underwent both smear techniques at a single visit; smear order was randomised and specimens were read blind. Both techniques utilised a Dacron swab, with water lubrication. Cytological specimens were prepared using a liquid based collection method (ThinPrep). The outcome measures were cytological specimen adequacy, cytological classification, presence of rectal columnar, squamous and metaplastic cells, contamination, patient comfort and acceptability, and volume of fluid that remained after the ThinPrep procedure.

Results: Regardless of smear order, guided smears were less likely to detect higher grade abnormalities than blind smears (15 v 27 cases, p = 0.001). Controlling for smear order, guided smears were more likely to be assessed as “unsatisfactory” for cytological assessment (OR 6.93, 95% CI 1.92 to 24.94), and contain fewer squamous (OR 0.20, 95% CI 0.04 to 0.94) and metaplastic cells (OR 0.12, 95% CI 0.03 to 0.54) than blind smears; there were no other statistically significant differences between techniques. Regardless of smear technique, first performed smears were more likely to detect a higher grade abnormality than second performed smears (23 v eight cases, p<0.001).

Conclusions: Blind cytology smears are superior to anoscope guided smears for screening for anal neoplasia in homosexual men.

I t is established that homosexual men have high rates of anal cancer1 and the presumed precursor lesion, anal intraepithelial neoplasia (AIN).2–5 These associations have led some to propose the screening of homosexual men by anal cytology smear,6 although the harms and benefits of screening are not well understood.

Past studies of AIN have used one of two techniques for the collection of anal cells, the “blind” method2 3 8–16 and the anoscope guided method.4 15–21 Anoscope guided sampling allows directed sampling from the ano-rectal transformation zone, the area of the anal canal where squamous carcinoma and its precursors most frequently develop.12 However, “blind” or non-guided sampling is the most widely used technique, typically physician collected rather than self collected.22 There is an extensive literature describing comparisons between blind “self sampling” and speculum directed cervical smears,23 but there has been little evaluation of physician collected anal smears. Unlike cervical smears, faecal contamination is an issue of concern with anal smears, with a resultant relatively high smear inadequacy rate.11 16 24 Furthermore, given the associations between anal human papillomavirus (HPV) infection and both anal cancer and AIN,3 16 25 the sampling technique must provide adequate cellularity for both cytology and ancillary tests such as HPV detection. We conducted a direct comparison of the two anal sampling techniques using a random sequence, paired design clinical trial.

METHODS

Study design

Two experienced clinicians, trained in the collection of anal cytology smears and anoscopy, enrolled participants and performed the anal smears. The anal canal of each participant was sampled using each technique at the same visit. The order in which the techniques were performed was randomly selected because of the unknown extent of any order effects. Smear order was pre-assigned in a sealed envelope that was opened only after informed verbal consent was established. Written consent was then obtained, and each participant completed a brief questionnaire about the recent use of their anus, and relevant medical history.

For both smears a Dacron swab moistened with water was rotated against the canal wall for 1 minute before being removed from the anal canal, shaken vigorously in liquid fixative PreservCyt (Cytyc Corporation) transport medium, and then discarded. During the “blind” smear the swab was also rotated against the canal wall during removal. For the anoscope guided smear a water lubricated clear plastic disposable anoscope was inserted 3–5 cm into the anal canal and adjusted with the aid of an external light source to reveal the transformation zone before sampling. During withdrawal of the anoscope the distal anal canal was inspected for warts or other abnormality and areas of interest were sampled. After smearing, participants rated the comfort and acceptability of each technique.

A cytopathologist processed the samples using the ThinPrep system (Cytyc Corporation) and then an experienced pathologist performed a conventional cytological assessment of the stained diagnostic cellular material. Anal cytology was classified using the Bethesda criteria for cervical cytology, with cross reference to the Australian Modified Bethesda system.

Abbreviations: AIN, anal intraepithelial neoplasia; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HGSIL, high grade squamous intraepithelial lesion; LGSIL, low grade squamous intraepithelial lesion; MSM, men who have sex with men
System. The cytologist and pathologist were blind to sample order and technique. The human research ethics committee at each participating institution approved the study.

Study population
Men who have sex with men (MSM) were recruited from a primary care clinic specialising in homosexual men’s health and a community based HIV testing and treatment centre. Consecutively presenting and referred MSM at least 18 years of age were approached. Men with a bleeding condition, and those taking anticoagulants (excluding aspirin/acetysalicylic acid), or with symptomatic anal disease, the severity or location of which would prevent taking of the smears, were ineligible.

Outcome measures
Smear specimens were compared with respect to adequacy for diagnostic cytology, contamination, the cell types present, the cytological classification, the sample volume remaining after the ThinPrep procedures, and participant comfort and acceptance.

Statistical analyses
The outcomes were separately examined by smear order and sampling technique using Stata (StataCorp, TX, USA). The t test was used for sample volume. For the categorical outcome variables, the extent of any effect by smear order and smear technique were separately assessed by comparing all categories of outcome data for paired samples. Where necessary, the categorical data were then dichotomised and paired samples were compared using McNemar’s χ² test. Conditional fixed effects logistic regression was then used to estimate the independent effects of smear order and technique, controlling for the other. In the regression analysis, smear adequacy was examined for all participants, and the remaining outcome variables were examined only for those participants with two adequate smears (n = 120).

RESULTS
Participant characteristics
The characteristics of the 151 MSM enrolled in the study are outlined in table 1. Seventy eight men underwent a “blind” smear followed by an anoscope guided smear; smear order was reversed for the remaining 73 participants. An anal cytological abnormality was detected in 62% of participants. HIV positive men were significantly more likely to have a cytology detected abnormality than HIV negative men (71% vs 49%, p = 0.03)

Cytology outcomes
Regardless of smear order, and ignoring cases with identical results for both smears, anoscope guided smears were more likely to be rated as inadequate for cytological assessment (20 vs three cases, p = 0.0004), less likely to detect a higher grade cytological abnormality (15 vs 27 cases, p = 0.001), less likely to contain a greater number of squamous cells (36 vs 48 cases, p = 0.02), and they were less likely to contain metaplastic cells (two vs 16 cases, p = 0.001), than “blind” smears.

Overall, “blind” smears “missed” one case of ASCUS, seven cases of LSIL and seven cases of HGSIL. Anoscope guided smears “missed” 10 ASCUS, nine LSIL, and eight HGSIL.

Regardless of smear technique, and ignoring cases with identical results for both smears, first performed smears were more likely to detect a higher grade cytological abnormality (23 vs eight cases, p < 0.001) and they were more likely to contain a greater number of squamous cells (66 vs 18 cases, p < 0.0001), than second performed smears.

Table 2 shows the independent effects of smear order and smear technique for the dichotomous outcome variables. After controlling for smear order, “blind” smears were significantly more likely to be rated as adequate for cytological assessment compared to anoscope guided smears, and they also contained significantly more squamous and metaplastic cells. There were no significant differences between first and second performed smears, after controlling for smear technique.

For satisfactory smears, there was no correlation between the presence of rectal glandular cells and the detection of anal dysplasia. Regardless of smear order or technique, anal dysplasia was detected in 71% of smears without any rectal cells and 64% of smears with some rectal cells.

Volume of fluid after ThinPrep analysis
There was no difference between first and second performed smears, or between “blind” and anoscope guided smears, in the volume of fluid remaining after ThinPrep preparation.

Comfort and acceptance and clinical findings
There were no significant differences in the reported comfort or acceptance of the two techniques, and both were well tolerated (table 3). Bleeding after the smear was more common with the anoscope than without (9% vs 4%). The

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| Table 1 Characteristics* of the 151 homosexual male participants |
|----------------|----------------|
| Age (years)    | 45 (11) (19–76) |
| HIV positive   | 95 (64%)        |
| Lowest CD4 count† (cells x10⁹/l) | 249 (186) (0–860) |
| Last CD4 count† (cells x10⁹/l)  | 580 (267) (120–1462) |
| Last plasma HIV-RNA load (copies/ml) | 13049 (30840) (50–122000) |
| Previous medical treatment of anus/canal | 61 (43%) |
| History of anal or genital warts† | 72 (50%) |
| Current anal or genital warts  | 24 (18%) |
| Last bowel movement (hours)    | 6 (7) (0–48) |

Anal cytology by worst case smear
- Inadequate samples 8 (5%)
- Negative 49 (32%)
- ASCUS 24 (16%)
- LGSIL (HPV, AINII) 41 (27%)
- HGSIL (AINIII, AINII) 29 (19%)

ASCUS, atypical squamous cells of undetermined significance; HGSIL, high grade squamous intraepithelial lesion; LGSIL, low grade squamous intraepithelial lesion.

*Data are mean (SD) (range) or proportions.
†For 95 HIV positive participants.
‡Available for 143 participants.
§Available for 134 participants.
transformation zone could not be located during the anoscope guided technique for 27% of participants; however, visualisation of the zone was unrelated to specimen adequacy.

**DISCUSSION**

We found that the “blind” smear is superior to the anoscope guided smear for sampling anal cells from MSM. Compared to anoscope guided smears, “blind” smears were significantly more likely to be rated as adequate for cytological assessment, and significantly more likely to contain squamous and metaplastic cells. This finding suggests that use of the anoscope may hinder anal cell collection, depending on its location in the canal, because access to the squamous epithelium distal to the transformation zone may be prevented. It is also possible that the insertion of the anoscope, with only water as a lubricant, may have caused some desquamation and contributed to the lower cellular yields. When analysed by detailed grades we also found that second taken smears were inferior at detecting higher grade abnormalities. This finding has potential implications for studies requiring multiple anal samples at a single visit.

The strengths of the study include the pre-assigned randomisation of smear order, the collection of both smears at the same visit, the blinded assessment of study outcomes by a single pathologist, and the use of identical processing procedures for both samples. However, we lost some ability to discriminate between paired smears because of between study variation in the criteria used to define a cytologically adequate sample, the sampling device (dry or moistened swab, wooden spatula, or brush), the duration of sampling, and the sample processing (conventional or liquid based).

The poorer performance of the anoscope guided smears in this study cannot be attributed to interference to the ThinPrep sample preparation system from lubricant applied to the anoscope, as only water was used. The most likely explanation is undersampling of the mucosa between the transformation zone and anal verge during removal of the anoscope.

Our study did confirm that in satisfactory smears the absence of rectal glandular cells was not correlated with the detection of anal neoplasia. This has also shown to be true for cervical screening specimens without endocervical glandular cells. There have been no previous studies of the effects of sequential sampling of the anal canal. Our data suggest an order effect when sampling from the anal canal, lending support to the current practice of taking an anal cytology smear before an anal swab for microbiological or molecular assessment. This finding also has potential implications for cervical screening and the use of two consecutive smears.

In conclusion, we found that the “blind” anal smear is superior to the anoscope guided anal smear for screening MSM for anal neoplasia. Our findings support the continued use of a cytology smear technique that is simple, quick, and relatively inexpensive to perform, and readily accepted by patients. However, the use of anoscopy may be clinically indicated by symptomatology and so should be considered after sampling for anal cytology in such cases.

### Table 2

<table>
<thead>
<tr>
<th>Description of outcome variable</th>
<th>“Blind” compared to guided smears*</th>
<th>Second compared to first smears*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear cytologically adequate†</td>
<td>6.93 (1.92 to 24.94)</td>
<td>0.87 (0.24 to 3.12)</td>
</tr>
<tr>
<td>Positive cytological diagnosis‡</td>
<td>1.67 (0.60 to 4.66)</td>
<td>0.48 (0.17 to 1.33)</td>
</tr>
<tr>
<td>No contamination of smear‡</td>
<td>1.51 (0.69 to 3.32)</td>
<td>0.76 (0.34 to 1.67)</td>
</tr>
<tr>
<td>No squamous cells detected‡</td>
<td>0.20 (0.04 to 0.94)</td>
<td>0.92 (0.18 to 2.75)</td>
</tr>
<tr>
<td>No rectal cells detected‡</td>
<td>0.64 (0.32 to 1.28)</td>
<td>1.28 (0.64 to 2.56)</td>
</tr>
<tr>
<td>No metaplastic cells detected‡</td>
<td>0.12 (0.03 to 0.54)</td>
<td>NA†</td>
</tr>
</tbody>
</table>

*Odds ratio (95% CI).
†Effect examined for all 151 participants.
‡Effect examined only for 120 participants with two adequate smears.
§Smears with a diagnosis of ASCUS, LGSIL or HGSIL were classified as positive.
*OR and 95% CI could not be calculated because of a zero cell.

### Table 3

<table>
<thead>
<tr>
<th>Description of procedure</th>
<th>Mean (range) rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blind (n = 150)</td>
</tr>
<tr>
<td>It was painful</td>
<td>2.0 (1–5)</td>
</tr>
<tr>
<td>It was uncomfortable</td>
<td>2.6 (1–5)</td>
</tr>
<tr>
<td>I felt at ease</td>
<td>3.9 (1–5)</td>
</tr>
<tr>
<td>I am prepared to have the procedure again</td>
<td>4.2 (1–5)</td>
</tr>
</tbody>
</table>

*Rated from 1 (strongly disagree) to 5 (strongly agree).
Key messages

- Blind smears are cytologically superior to anoscope guided smears for screening for anal intraepithelial neoplasia in homosexual men
- A clinic based sample of homosexual men reported no difference in comfort between blind and anoscope guided anal smears
- Second taken anal smears are inferior at detecting higher grade anal abnormalities

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CONTRIBUTORS

GM, CMV, and AEG conceived and designed the study; CMV coordinated the study, performed the data analysis, and wrote the initial draft; JSA and RHJ recruited participants, performed the clinical procedures, provided demographic and behavioural data, and made comments on drafts; GM performed the cytology and made comments on drafts; AEG made comments on drafts.

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