

A study of cellular counting to determine minimum thresholds for adequacy for liquid-based cervical cytology using a survey and counting protocol

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Scientific summary

Minimum thresholds for adequacy for LBC

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Background

Liquid-based cytology (LBC) has been implemented across the UK Cervical Screening Programme following a National Institute for Health and Care Excellence (NICE) recommendation in 2003. There are two different cytology systems, SurePath™ (SP; BD Diagnostics, Burlington, NC, USA) and ThinPrep™ (TP; Hologic, Inc., Bedford, MA, USA), both of which are used in the UK. The NICE report highlighted the need for a definition of cell adequacy, following which a study designed to address this was commissioned by the National Institute for Health Research Health Technology Assessment programme. Previously reported studies on this topic have lacked sufficiently robust evidence on which to base the necessary practice guidelines. The Bethesda System guidance has recommended a minimum acceptable cell count (MACC) for the TP system of 5000 cells; however, this was reached in a rather arbitrary way and practice varies. As both systems are extensively employed in the NHS Cervical Screening Programme (NHSCSP), it was necessary that the required study evaluate both systems. This report describes the results of the study, which include a survey of current practice across 56 laboratories; investigations into the reliability of cell counting; the relationship between cell counts and slide reporting across these 56 laboratories; and the impact of serial dilution on slide reading. The aim of this study was to achieve a reliable basis on which recommendations could be developed for MACCs for both TP and SP.

Objectives

The objectives of this study were (1) to conduct a survey across the country of cytology laboratories in order to assess current standard procedures and practice for designating LBC slides inadequate; (2) to use a standardised counting method to achieve a reproducible basis of establishing the cellularity of a LBC sample; (3) to correlate the classification of reported slides (inadequate, negative or abnormal) among slides of known cellularity; and (4) to evaluate the impact of varying cellularity through dilution on the likelihood of detecting abnormal cells.

Methods

The overall study was divided into four separate components to address the objectives:

1. Current standard procedures and practice were determined through a questionnaire-based survey of 56 cytology laboratories in England, Wales and Scotland and through review of submitted existing protocols.
2. A counting protocol was developed by three experienced cytopathologists who each counted cells on a sample of routinely obtained slides to allow a comparison of counts using alternative starting points for cell counting in adjacent high-power fields. The total cell count was computed as (mean cell count of 10 high-power fields) × (area of cell deposit) ÷ (area of ocular). This allowed interobserver variation and the reproducibility of counts to be assessed, in order to select an optimal standard counting protocol.
3. A slide set was generated from all participating laboratories by requesting 20 slides from each of the inadequate, mild dyskaryosis and high-grade dyskaryosis classifications and a further 50 slides classed as negative. These were batched and recirculated to the network of 56 laboratories within the NHSCSP, Scottish Cervical Screening Programme and Cervical Screening Wales that had been recruited to survey. Cell counts were performed by designated staff in each laboratory using the standard counting protocol and counts were correlated with the reported slide classification (inadequate, negative, low or high grade).

4. Two methods of dilution were employed on cell samples containing dyskaryotic cells, in order to study the impact of reducing cellularity on the detection of cytological abnormalities. The first method was simple fluid dilution, termed unmixed dilution, and the second method involved mixing the sample with normal cells in order to retain overall cellularity but reduce the proportion of abnormal cells. The first method was intended to mimic overall hypocellular slides and the second method to mimic slides in which a diminishing number of abnormal cells were present within the entire range of cellularity.

Results

The survey of cervical screening laboratories included 28 SP and 28 TP laboratories and, of these, all of the SP laboratories and 27 TP laboratories responded. Practice was found to be variable; specifically, 15 out of 27 TP laboratories and 18 out of 28 SP laboratories used morphological criteria to determine slide adequacy, whereas 7 out of 27 TP laboratories and 13 out of 28 SP laboratories recorded indicators of transformation zone sampling, including both endocervical cells and metaplastic squamous cells. All but one of the SP laboratories assessed specimen adequacy by means of a MACC, and of the 11 out of 28 SP laboratories that specified a MACC figure, all stated a minimum of 15,000. Among the TP laboratories, 20 out of 27 assessed MACC by cell counting, and for the 11 out of 27 laboratories that specified the MACC figure, it ranged between 5000 and 15,000. Of the laboratories that responded to the survey, 29 out of 55 submitted standard operating procedures (SOPs).

The exercise to assess reliability of cell counting demonstrated a kappa value for SP of 0.851 [95% confidence interval (CI) 0.787 to 0.915] for counts performed in the same starting position and 0.906 (95% CI 0.804 to 1.00) in a different starting position. The corresponding TP figures were 0.614 (95% CI 0.461 to 0.767) and 0.590 (95% CI 0.407 to 0.774) for the same and different starting positions, respectively. There was, therefore, no significant difference between starting positions of counting but SP showed stronger interobserver agreement than TP. Numerically, there were very few instances of substantial disagreements in cell counts.

The cell counting survey from all participating laboratories amounted to 3110 SP slides and 3176 TP slides. The mean cell counts for inadequate samples were around 14,000 and 11,000 for SP and TP, respectively. A one-way analysis of variance (ANOVA) revealed a significant difference in these counts between SP laboratories but not between TP laboratories.

The cell counts for samples assessed as inadequate were far lower than the mean counts for negative, low- and high-grade abnormal samples, which averaged around 50,000. Of the SP slides submitted, 75% of inadequates had cell counts of less than 15,000, and only 2.5%, 2.3% and 1.4% of the high-grade, low-grade and negative samples, respectively, were below this count. A MACC set at 15,000 for SP would, therefore, achieve a sensible balance between sensitivity to detect cytological abnormalities and the maintenance of low rates for inadequate samples. With regard to TP, 43% of inadequate slides had cell counts of less than 5000 and only 1.8%, 1.6% and 1.3% of high-grade, low-grade, and negative samples, respectively, were below this count. Therefore, a MACC set at 5000 for TP would achieve a similar balance between sensitivity and inadequate rates.

The dilution study to vary slide cellularity involved 2400 slides of increasing dilution, which were each sent to three laboratories from a panel of 24. The overall kappa coefficient for slides of all categories was very similar for SP and TP at 0.593 (95% CI 0.571 to 0.610) and 0.609 (95% CI 0.589 to 0.633), respectively. For both systems the kappa value for the inadequate and the 'low- and high-grade'-combined samples was higher than for negative and high-grade samples. In the unmixed dilutions, there was evidence that for SP the detection of either low- or high-grade abnormalities increased as cellularity increased from 75% (95% CI 59.2% to 86.1%) at a cellularity of between 5000 and 10,000, to 91.7% (95% CI 87.4% to 94.6%), at a cellularity of over 50,000. For the unmixed dilutions in TP, there was a similar observation with cellularity below 2500 showing a detection of 75.8% (95% CI 55% to 88%) rising significantly to

98.2% (95% CI 91.5% to 99.7%) at cellularity between 50,000 and 75,000. The lowest 10% of cellularity (< 15,000 for SP and < 5000 for TP) had a significantly lower detection of abnormalities in both systems than the middle 80% (up to 58,000 for both SP and TP).

In the mixed-dilution study, it was shown that, as the number of dyskaryotic cells was diluted by the number of normal cells, the likelihood of detecting abnormalities was reduced significantly in SP but not significantly in TP. As the actual number of dyskaryotic cells decreased so did the likelihood of detection. Compared with a reference standard of greater than 50 dyskaryotic cells on slides above the MACC, the odds ratio for detection when fewer than 25 dyskaryotic cells were present was 0.49 and 0.74 for SP and TP, respectively.

Conclusions

Each section of this study has yielded the basis for reliable conclusions which have implications for laboratory practice within the NHSCSP. There is quite variable practice in classifying slides as inadequate, and it would be beneficial to agree a standard procedure for cell counting, and a standard MACC for both SP and TP. The counting SOP specified in this report showed good reproducibility and could be widely adopted. It was clear that there is a very wide range of cell counts for slides classified as inadequate, some of which are a result of factors other than hypocellularity. The evidence from this study suggests that a MACC of 15,000 and 5000 for SP and TP, respectively, would probably achieve the best balance between maintaining low levels of inadequate slides and not compromising on the chances of detecting abnormalities. Many inadequate slides had counts above this recommended MACC. However, a MACC threshold above which there would be few inadequate samples would risk a significant rise in the proportion of inadequate slides reported, without any evidence of improved detection of abnormal cells. Finally, there is evidence that in slides with a normal range of cell counts, slides with fewer than 25 dyskaryotic cells are associated with a reduced chance of detecting abnormalities compared with slides with more than 50 dyskaryotic cells, and this may have implications for medicolegal cases where missed abnormalities have resulted in the development of cancer.

Future work

The findings of this study should inform the development of laboratory practice guidelines.

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