Liquid based cytology: examination of its potential in a chlamydia screening programme

J Hopwood, H Mallinson, E Hodgson, L Hull

Objective: To assess the feasibility of testing for chlamydia directly on a single liquid based specimen (ThinPrep test) collected for cervical screening.

Method: Cervical smears were taken using a Cervex spatula and rinsed in the liquid based cytology collection vial. Following this, the conventional sample for chlamydia testing was taken from the endocervix using an Abbott Collection kit. Cytological specimens were prepared using an automated slide processor. Residual cellular material and the conventional samples were sent to the laboratory where both were tested for chlamydia by ligase chain reaction (LCR). The manufacturer’s protocol for LCR urine testing was modified to substitute 1 ml of PreservCyt suspension.

Results: 581 women had both swab and cytology suspension tested for Chlamydia trachomatis with LCR. There were 19 concordant positive and 562 concordant negative reports. The stability of chlamydia in the cytology suspension was maintained for at least 5 months.

Conclusion: The findings lead us to conclude that samples collected for liquid based cytology using the ThinPrep test collection vial provide a potential platform for chlamydia screening, though the study established several issues to be addressed to make this a practical proposition.

The advent of liquid based cytology (LBC) provides a potential platform for chlamydia screening, though the study established several issues to be addressed to make this a practical proposition.

The Department of Health funded a pilot screening programme (August 19 to September 2000) in Wirral and Portsmouth using a urine ligase chain reaction (LCR) test. Women were offered testing in general practice, family planning clinics or going for their first cervical smear be offered chlamydia screening.

The Department of Health funded a pilot screening programme to use LBC for colposcopy in Wirral’s colposcopy service chlamydia testing is routinely offered to all women under 30 years. Chlamydia infection may contribute to the cytological abnormality which may resolve after its treatment; it could be the cause of a clinical referral such as abnormal bleeding or appearance of the cervix; it could result in treatment morbidity.

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METHODS

Patients and specimen collection

Abbreviations: LBC, liquid based cytology; LCR, ligase chain reaction
service, Arrowe Park Histopathology Department, Liverpool Public Health Laboratory (PHL) and the Wirral Chlamydia Office. Information sheets for patients and a protocol were developed and ethical approval obtained. From May 2001 to May 2002, women attending for colposcopy were given the information and those having a smear as part of diagnosis or follow up were offered the dual testing of cervical smear and chlamydia in addition to a conventional endocervical swab for chlamydia testing.

Cervical smears were taken using a Cervex spatula rotated five times and vigorously rinsed in the bottle containing PreservCyt fluid. Following this, the conventional sample for chlamydia testing was taken from the endocervix using an Abbott LCx swab collection kit.

A linked request for a chlamydia test on the suspension was transmitted to the cytology laboratory by attaching a chlamydia reference number (CRN) from the specially designed microbiology request form (RF) to the standard cervical smear request form HMR/101/5. The bottles with HMR101 together with the swabs in the microbiology RF bag were checked for correct identification and taken to the cytology laboratory daily.

Throughout the process, standard procedures were in place to prevent cross contamination of all samples. No extra measures were introduced.

Sample preparation and transport

The LBC specimen vials along with their accompanying swabs in microbiology RF/bag and appropriate forms were taken to the cytology laboratory (Arrowe Park Hospital Cytology Laboratory) for processing. Samples were checked into the laboratory and because the currently used computer program was not designed to manage this, a day book was kept. A record was kept of the order the samples went into the machine to enable monitoring of results following a positive chlamydia test.

Cytological specimens were prepared using an automated slide processor (ThinPrep 2000 System, Cytyc UK Ltd, Crawley, Sussex) according to the manufacturer’s specifications. Slides were Pap stained, screened for cellular abnormalities, and reported according to institutional procedures. There are no national guidelines for reading and reporting LBC smears, so for this study senior medical scientists both screened and checked the smears, though for traditional smears they would not carry out the first screen.

The endocervical swabs in microbiology RF bags were placed in the refrigerator.

Only when it was determined that there was satisfactory cellular content in the smear, was the residual material in the PreservCyt fluid in its container placed in a second microbiology RF linked to the one containing the swab.

These samples were sent in existing transport arrangements to Liverpool PHL. There, both swabs and suspension were tested for chlamydia by LCR (LCx Probe System, Abbott Laboratories, Abbott Park, IL, USA). The manufacturer’s protocol for urine which utilises a centrifuged deposit was modified to substitute 1 ml of PreservCyt cell suspension as protocol for urine which utilises a centrifuged deposit was modified to substitute 1 ml of PreservCyt cell suspension as specimen for LBC smear processing through the ThinPrep 2000 Processor followed processing of another patient’s fluid with a negative chlamydia result. This leads us to assume there was not an issue of cross contamination during the slide preparation stage.

The stability of chlamydia in positive fluids was examined by retesting 16 of the LBC specimens after they had been held at ambient temperature for several months. All of 16 re-tested up to the time of reporting have remained positive by LCR for at least 5 months.

**RESULTS**

During the 12 month period from May 2001 to May 2002, 581 women had both swab and suspension tested for *Chlamydia trachomatis* with LCR. In total, there were 19 concordant positive and 562 concordant negative reports.

For the first 2 months, women of all ages were offered the test. There were no positive results in women over 30 years old. This finding is consistent with previous chlamydia prevalence studies and therefore, screening was then restricted to women under 30 years old.

One case was reported negative following a pattern of testing that showed an LCR negative fluid but some initial reactivity of the LCR swab. Two cases were reported negative following a pattern of testing that showed an LCR negative swab but some initial reactivity of the LCR on fluid. Follow up PCR was negative for all three cases. Such patterns were known to occur with the then current batches of LCR kits.12 For the two fluids initially reactive but reported negative, processing through the ThinPrep 2000 Processor followed processing of another patient’s fluid with a negative chlamydia result. This leads us to assume there was not an issue of cross contamination during the slide preparation stage.

The stability of chlamydia in positive fluids was examined by retesting 16 of the LBC specimens after they had been held at ambient temperature for several months. All of 16 re-tested up to the time of reporting have remained positive by LCR for at least 5 months.

**DISCUSSION**

In this direct comparison of testing for *C. trachomatis* specimens collected using traditional swab with specimens collected in PreservCyt cytology collection medium we found no discrepant cases between the two methods. In addition, given the 100% concordance and the fact that each of the 19 positive cases was followed by a negative case during the slide- processing phase, we have no evidence of cross contamination using the ThinPrep 2000 processor.

These findings lead us to conclude that samples collected for fluid based cytology using the ThinPrep Pap test collection vial provide a viable platform for chlamydia screening.

Our findings are consistent with previous studies suggesting that chlamydia screening is best applied to women under 30 years of age. As noted, since during the first 2 months of the study there were no positive results in women over 30, we restricted our study to women under 30.

Even though we restricted our population to younger women, we found a lower chlamydia positivity than we had ever experienced in the colposcopy setting. However, in this study testing took place at all visits, which include assessment, treatment, and follow up, so some women had already attended and been tested and managed if positive. Also, it is likely that the active screening programme throughout Wirral meant that many of those referred had already been screened in general practice. Further examination showed that where we could establish the status of the visit 9/108 (8.3%) tested positive at their first visit, and 2/175 (1.7%) tested positive at subsequent visits. We believe this supports the premise that the lower than expected overall prevalence of chlamydia infection in this population is attributable to previous detection and treatment.

One option of an equitable opportunity for women to have a chlamydia test, which would facilitate a community message and inform about other testing options is to send information about chlamydia with the first smear invitation.
This gives an opportunity for an informed decision about the test. A request for chlamydia testing can be made clearly via the smear request form, in this study by using adhesive chlamydia labels.

If LBC is used, then this study confirms that use of its residual suspension would be possible for chlamydia testing. The LCR modified urine test on PreservCyt performed well. There seems to be excellent stability of chlamydia in the fluid so that transport and storage would not be a restricting factor, but clearly any backlog affecting cytology preparation could potentially lead to an unacceptable delay in testing for chlamydia where timely treatment is all important. This study involved some change of practice in the cytology laboratory to expedite sending of samples to the microbiology laboratory.

In a colposcopy setting in this study no special measures were required for safe storage of these small volumes of flammable liquid but in larger scale programmes this may need to be addressed by both clinics and laboratories.

Results and management of those who screened positive were dealt with by the colposcopy service according to current practice. However, there is not a nationwide system that could support this in a community based programme. The current call-recall mechanism for the NHS cervical screening programme could not manage the recording of dual tests and results. However, in Wirral where there is a well defined support system for chlamydia screening, dual testing could readily be managed. Despite this logistical difficulty, the other advantages that could facilitate delivery of an equitable programme suggest that further work on this is required.

Note
Since this study there is NICE approval for LBC in the cervical screening programme (www.nice.org.uk). Although it is now recommended that cervical screening should commence at 25 years rather than 20 years, the time of first smear may still represent an opportunity for those who have never accessed or been offered a chlamydia test previously and this could even have the potential to contribute to the measure of uptake and coverage of the chlamydia screening programme (Sasieni P, Adams J, Cuzick J. Benefits of cervical screening at different ages; evidence from the UK audit of screening histories. British Journal of Cancer July 2003, www.cancerscreening.nhs.uk ages;evicen)

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