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# Assessment of two methods for rapid intrapartum detection of vaginal group B streptococcal colonisation

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## Abstract

***Aims*—To compare two methods for the rapid detection of intrapartum vaginal carriage of group B streptococci (*Streptococcus agalactiae*) with standard culture techniques and to establish their suitability for routine use.**

***Methods*—Vaginal swabs from 266 patients in labour were incubated in glucose broth in an anaerobic atmosphere for four to six hours. The Wellcogen Strep B latex particle agglutination test kit was subsequently used for antigen detection. In the second part of the study swabs from 117 women were assessed for the presence of group B streptococci using the ICON STREP B immunoconcentration assay (Hybritech). Both methods were compared with standard semiquantitative culture on Columbia horse blood agar and Islam's medium.**

***Results*—In the first study vaginal carriage of group B streptococci was shown in 38 of 266 (14·3%) patients by culture. Latex particle agglutination with the Wellcogen kit detected 30 of these positive results (sensitivity 78·9%, specificity 100%). In those patients with moderate to heavy colonisation (> $10^4$ colony forming units per millilitre) antigen was detected in all (26/26) culture positive patients (sensitivity 100%, specificity 100%). In the second study 16 (13·7%) patients were culture positive. The ICON test detected 11 positive results (sensitivity 68·8%, specificity 100%) and for heavy colonisation ($10^5$ cfu/ml) detected nine of nine cases (sensitivity 100%, specificity 100%). The ICON test took 10 to 15 minutes to perform.**

***Conclusion*—These tests are potentially useful for the rapid detection of group B streptococci vaginal colonisation in labour, particularly heavy colonisation. Both tests are insufficiently sensitive to replace standard culture methods.**


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Recent editorials in two leading journals have discussed the need for more rapid and reliable methods of intrapartum detection of vaginal carriage of group B streptococci.[1 2] This organism remains an important cause of neonatal sepsis, causing significant mortality and morbidity, much of which is preventable.[3] Ultimately an effective vaccine may become available,[4] but until then antibiotics are the main means of limiting disease.

Chemoprophylaxis during labour is of undoubted benefit,[5–9] but the problem is one of patient selection for such prophylaxis. Guidelines for chemoprophylaxis have recently been issued in the USA.[10] Known carriers of group B streptococci with defined risk factors (maternal fever during labour, premature or prolonged rupture of membranes, multiple pregnancy or premature labour) are recommended to receive prophylaxis. Universal screening (by culture) during pregnancy will identify many carriers, but carriage is unstable. Some women found to be negative by screening during pregnancy will be positive at term, while others may receive insufficient antenatal care to be screened at all. Overreliance on antenatal screening will therefore place some women at continued risk. Selective screening has been shown to be unhelpful.[11] The ideal time to screen is during labour, but conventional screening at this time is too slow if results are to guide intrapartum prophylaxis. Quicker results are needed.

Many published papers demonstrate the continuing search for a reliable test,[12] but no test has yet become established. This paper reports the results of a comparison of two rapid methods with standard culture for intrapartum detection of vaginal group B streptococcal carriage.

## Methods

The study was divided into two parts: part A involved enrichment of vaginal swabs, followed by latex particle agglutination (LPA), using the Wellcogen Strep B test (Wellcome Diagnostics, Dartford, Kent). Part B involved enrichment followed by use of the ICON STREP B test (Hybritech Europe SA, Liège, Belgium). Low vaginal swabs were obtained by the attending midwife, after informed consent, from women presenting to the delivery suite in labour at Homerton Hospital, Hackney, over a four month period. For the second part of the study duplicate swabs were taken from each patient. After sampling the cotton-tipped swabs were placed into Amies transport medium and sent to the laboratory.

### PART A

Swabs were inoculated on to non-selective Oxoid Columbia horse blood agar and Islam's medium[13] (five swabs per plate plus a positive control organism). Each swab was

then inoculated into 3 ml 0·25% glucose broth (Southern Group). The tips of the swabs were cut and left in the broth. Plates were incubated at 37°C for up to 48 hours in an anaerobic atmosphere, and were examined for haemolysis or pigment production at 24 and 48 hours. Orange pigment production on Islam's medium was taken as evidence of presumptive group B streptococci. Haemolytic colonies were tested with the Streptex Lancefield grouping kit (Wellcome Diagnostics, Dartford, Kent) for group B streptococcal antigen. Quantitative growth of group B streptococci on the primary plates was assessed as follows.

| *Semi quantitative assessment* | *Colony forming units* |
|---|---|
| 0 | 0 |
| 1+ | <10 |
| 2+ | 10–50 |
| 3+ | >50 (or confluent growth) |

The glucose broths were incubated at 37°C in an anaerobic atmosphere with the tops loosened so that maximum anaerobic conditions were obtained, for four to six hours. Swab tips were then removed and discarded. Each broth was then subcultured on to Islam's medium and incubated anaerobically. The broths were boiled in a water bath for five minutes and then centrifuged for five minutes at 3000 rpm. Latex testing of the supernatant fluid was then performed using the Wellcogen Strep B test (Wellcome Diagnostics). Thirty microlitres of broth was mixed with about 5 $\mu$l (1 loopful) of latex suspension on a glass tile and the tile was rotated for up to two minutes. A control latex was used to eliminate samples causing autoagglutination. Positive results were taken as those which caused visible agglutination within two minutes.

*Table 1 Comparison of plate counts from known group B streptococcal concentrations*

| *Colony count* | *Grade* | *Equivalent group B streptococcal concentration (cfu/ml)* |
|---|---|---|
| <10 | 1+ | $10^3$ |
| 10–50 | 2+ | $10^4$ |
| >50 | 3+ | $10^5$ |

*Table 2 Comparison of culture and LPA after pre-enrichment*

| *Group B streptococci culture result* | *No of patients* | *LPA positive** | *% LPA positive* |
|---|---|---|---|
| 0 | 228 | 0 | 0 |
| 1+ | 12 | 4 | 33 |
| 2+ | 7 | 7 | 100 |
| 3+ | 19 | 19 | 100 |

*LPA = Latex particle agglutination test

For all culture positive results:
Sensitivity = 78·9%
Specificity = 100%
Positive predictive value = 100%
Negative predictive value = 96·6%

For moderate to heavily colonised patients (2 + or 3 +, > $10^4$ cfu/ml):
Sensitivity = 100%
Specificity = 100%
Positive predictive value = 100%
Negative predictive value = 100%

PART B

Duplicate swabs were collected from each patient—one swab was processed as in part A. The second swab was processed using the ICON STREP B immunoconcentration assay as per the manufacturer's instructions. This test involves an enzyme immunoassay, with labelled anti-group B streptococcal antibody bound to a porous membrane within an ICON cylinder. Each swab was subjected to a nitrous acid antigen extraction procedure; the extract was mixed with alkaline phosphatase-conjugated anti-group B streptococcal antibody. The filtered mixture was passed through the membrane containing bound group B streptococcal antibody, in the ICON cylinder. After washing and addition of enzyme substrate a colour change was seen in the centre of the ICON membrane if labelled group B streptococcal antibody-antigen complex had been bound to the membrane. A colour change of any intensity was taken as positive. Results were read after two minutes and designated either positive or negative—no grading of the result was possible.

An assessment of the sensitivity of these tests was performed by artificially inoculating swabs into broths containing serial dilutions of group B streptococci, prepared from an overnight culture, or by inoculating swabs with 100 $\mu$l of each broth dilution. These were plated out, incubated, and colony counts graded as for clinical swabs. Broth viable counts were calculated by a spread plate method. A comparison of colony counts on swab plates with known group B streptococcal concentrations was made.

## Results

A total of 266 patients were tested in part A of the study; 117 of these were also studied in part B. A total of 38 of 266 (14·3%) patients were positive for group B streptococcal vaginal carriage by culture. For part B alone, 16 of 117 (13·7%) patients were culture positive.

There were no cases of sepsis due to group B streptococci in infants born to mothers enrolled in the study.

In assessing the sensitivity of plate cultures, results were similar for both arms. Comparisons are shown in table 1.

PART A

Thirty of 38 patients with group B streptococcal carriage detected by culture were detected by LPA testing after anaerobic pre-incubation in broth. There were no false positive results. Results are shown in table 2.

PART B

Eleven of 16 culture positive patients were detected using the ICON test. There were no false positive results. Results are shown in table 3.

## Discussion

Many rapid tests for the detection of group B streptococci have been studied, including

*Table 3 Comparison of culture and ICON immunoassay*

| *Group B streptococci culture result* | *No of patients* | *ICON positive* | *% ICON positive* |
|---|---|---|---|
| 0 | 101 | 0 | 0 |
| 1+ | 5 | 1 | 20 |
| 2+ | 2 | 1 | 50 |
| 3+ | 9 | 9 | 100 |

For all culture positive patients:
Sensitivity = 68·8%
Specificity = 100%
Positive predictive value = 100%
Negative predictive value = 95·3%

For heavily colonised patients (3+, > $10^5$ cfu/ml):
Sensitivity = 100%
Specificity = 100%
Positive predictive value = 100%
Negative predictive value = 100%

Gram stains, LPA tests, coagglutination, pigment production, enzyme immunoassays (EIA) and DNA probes, without any one test becoming established as sufficiently reliable or sensitive.[12] Most show improved sensitivity for heavily colonised patients.

Latex particle agglutination tests have been studied by many investigators, although most have looked at direct antigen extraction from vaginal swabs, followed by LPA.[12 14 15] A few have studied broth pre-enrichment followed by LPA. Stiller *et al*, using the Streptex kit, showed a sensitivity of 91·8% after eight to 12 hours of pre-incubation in Todd-Hewitt broth (specificity 97·6%, positive predictive value 89·6%, negative predictive value 98·2%).[16] The Wellcogen kit has also been studied, demonstrating 86·8% sensitivity after eight hours of pre-incubation, which increased to 94·7% after nitrous acid extraction of centrifuged pellets.[17] Staphylococcal coagglutination tests have been reported to have low sensitivity after pre-enrichment.[12 18] Our experience with the Wellcogen test shows that it is highly specific and has excellent sensitivity for moderate to heavy colonisation after four to six hours of anaerobic pre-enrichment, a shorter time than has been studied before. The sensitivity of the test, down to at least $10^4$ cfu/ml, is particularly noteworthy and makes it very attractive. However, this test, while being simple and very reliable, is only suitable for performance within a laboratory setting rather than at the bedside. The time taken to achieve a result is still a drawback, both in terms of rapidity and out of hours work, but a useful result could be provided during the period of labour in many cases. In a Finnish study, using an LPA test after pre-enrichment for five to seven hours, only 38% of patients had delivered before a test result could influence management.[19]

There have been two previous reports of experience with the ICON STREP B test.[20 21] Gentry *et al*, in a study of 300 patients in labour, compared the ICON with culture on blood agar.[20] They showed a sensitivity of 33%, a specificity of 95%, a positive predictive value of 43% and a negative predictive value of 93%. For heavily colonised patients, defined in this study as greater than $10^5$ cfu/ml of vaginal fluid, the sensitivity was 100%. Armer *et al* showed a sensitivity for the ICON of only 11% overall in a study of 182 patients (specificity 100%, positive predictive value 100%, negative predictive value 78%).[21] The sensitivity was 100% for heavily colonised patients, again defined as > $10^5$ cfu/ml. This latter study used broth enrichment, in addition to culture, for comparison,[21] and thus detected many more culture positive cases (23%) than either the previous study (10%)[20] or our investigation. This probably accounts for the much lower overall sensitivity. Both these previous studies estimated heaviness of colonisation by inoculating swabs with an arbitrary 100 $\mu$l of broth containing group B streptococci at known titres and plating these out as for clinical swabs. This may have resulted in overestimates of the density of group B streptococci in vaginal fluid. However, the limit of sensitivity seemed to be similar in both reports, and our findings suggest that 100 $\mu$l per swab gives similar results to direct inoculation into broth. Our results confirm the findings of these two studies, demonstrating that the ICON has a very high sensitivity and specificity for heavily colonised patients. It failed to detect some more lightly colonised patients, some of whose infants will become colonised, so it could not replace standard culture methods for detection of group B streptococci. However, the risk to an infant of group B streptococcal colonisation and sepsis is directly related to the heaviness of maternal vaginal carriage,[22 23] so detection of heavily colonised mothers alone is of value. We also found that the ICON test had a high positive predictive value, unlike Gentry *et al*,[20] but confirming the findings of Armer *et al*.[21] The ICON test is easy to interpret and the above findings, together with the short time to produce a result, indicate that this test is potentially very useful as a tool for rapid testing, as an adjunct to plate culture. However, although rapid, there are several steps involved in performing the test, thereby possibly limiting its use to trained personnel and hence its usefulness as a bedside test.

Costs are an inevitable consideration. The Wellcogen Strep B test costs £46 (+ VAT) (at 1993 prices) and provides sufficient reagent for 200 tests by this method (£0·23/test), but the main expenses are staff costs in providing a test that takes up to six hours to perform, especially for out of hours provision. The ICON STREP B test costs £65 (+ VAT) for 20 tests (£3·25/test), but offers considerable savings in terms of laboratory staff time. Local considerations must influence which of these tests a laboratory might decide to offer.

Both of these tests are highly sensitive and specific for patients heavily colonised with group B streptococci. The ICON STREP B immunoconcentration assay is very quick and relatively simple. The Wellcogen Strep B test, although taking up to six hours with pre-enrichment, was more sensitive. Neither of these tests is sufficiently sensitive to replace standard culture methods, but both could be of value for rapid detection of maternal group B streptococcal carriage during labour.

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1 van Oppen C, Feldman R. Antibiotic prophylaxis of neonatal group B streptococcal infections. *BMJ* 1993; **306**:411–2.
2 Wessels MR, Kasper DL. The changing face of Group B streptococcal disease. *N Engl J Med* 1993;**328**:1843–4.
3 Fischer G, Horton RE, Edelman R. Summary of the National Institutes of Health Workshop on group B streptococcal infection. *J Infect Dis* 1983;**148**:163–6.
4 Feldman RG, Fleer A. The immune response to the group B streptococcus. *Rev Med Microbiol* 1992;**3**:52–8.
5 Yow MD, Mason EO, Leeds LJ, Thompson PK, Clark DJ, Gardner SE. Ampicillin prevents intrapartum transmission of group B streptococcus. *JAMA* 1979;**241**: 1245–7.
6 Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. *N Engl J Med* 1986;**314**:1665–9.
7 Boyer KM, Gadzala CA, Kelly PD, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. III. Interruption of mother-to-infant transmission. *J Infect Dis* 1983;**148**: 810–5.
8 Morales WJ, Lim DV, Walsh AF. Prevention of neonatal group B sepsis by the use of a rapid screening test and selective intrapartum chemoprophylaxis. *Am J Obstet Gynecol* 1986;**155**:979–83.
9 Easmon CSF, Hastings MJG, Deeley J, Bloxham B, Rivers RPA, Marwood R. The effect of intrapartum chemoprophylaxis on the vertical transmission of group B streptococci. *Br J Obstet Gynaecol* 1983;**90**:633–5.
10 American Academy of Pediatrics, Committee on Infectious Diseases, Committee on the Fetus and Newborn. Guidelines for prevention of group B streptococcal (GBS) infection by chemoprophylaxis. *Pediatrics* 1992;**90**:775–8.
11 Regan JA, Klebanoff MA, Nugent RP. The epidemiology of group B streptococcal colonization in pregnancy. *Obstet Gynecol* 1991;77:604–10.
12 Yancey MK, Armer T, Clark P, Duff P. Assessment of rapid identification tests for genital carriage of group B streptococci. *Obstet Gynecol* 1992;**80**:1038–47.
13 Islam AKMS. Rapid recognition of group-B streptococci. *Lancet* 1977;**i**:256–7.
14 Quentin R, Dubarry I, Gignier C, Saulnier M, Pierre F, Goudeau A. Evaluation of a rapid latex test for direct detection of Streptococcus agalactiae in various obstetrical and gynaecological disorders. *Eur J Clin Microbiol Infect Dis* 1993;**12**:51–4.
15 Green M, Dashefsky B, Wald ER, Laifer S, Harger J, Guthrie R. Comparison of two antigen assays for rapid intrapartum detection of vaginal group B streptococcal colonization. *J Clin Microbiol* 1993;**31**:78–82.
16 Stiller RJ, Blair E, Clark P, Tinghitella T. Rapid detection of vaginal colonization with group B streptococci by means of latex agglutination. *Am J Obstet Gynecol* 1989;**160**:566–8.
17 Teti G, Burdash NM, Corey K, Fava C. Detection of group B streptococci by agglutination testing from selective broth cultures. *Ann Clin Lab Sci* 1985;**15**:292–8.
18 Jones DE, Friedl EM, Kanarek KS, Williams JK, Lim DV. Rapid identification of pregnant women heavily colonized with group B streptococci. *J Clin Microbiol* 1983;**18**:558–60.
19 Tuppurainen A, Hallman M. Prevention of neonatal group B streptococcal disease: intrapartum detection and chemoprophylaxis of heavily colonized parturients. *Obstet Gynecol* 1989;**73**:583–7.
20 Gentry YM, Hillier SL, Eschenbach DA. Evaluation of a rapid enzyme immunoassay test for detection of group B Streptococcus. *Obstet Gynecol* 1991;**78**:397–401.
21 Armer T, Clark P, Duff P, Saravanos K. Rapid intrapartum detection of group B streptococcal colonization with an enzyme immunoassay. *Am J Obstet Gynecol* 1993;**168**:39–43.
22 Jones DE, Kanarek KS, Lim DV. Group B streptococcal colonization patterns in mothers and their infants. *J Clin Microbiol* 1984;**20**:438–40.
23 Boyer KM, Gadzala CA, Kelly PD, Burd LI, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures. *J Infect Dis* 1983;**148**: 802–9.