Diagnostic research in perspective: examples of retrieval, synthesis and analysis
Bachmann, L.M.

Citation for published version (APA):
Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: systematic review

Honest Honest, Lucas M. Bachmann, Janesh K. Gupta, Jos Kleijnen, Khalid S. Khan

BMJ. 2002 Aug 10;325(7359):301
Chapter 4

Abstract

Objective: To determine the accuracy with which cervico-vaginal fetal fibronectin test predicts spontaneous preterm birth in asymptomatic and symptomatic pregnant women.

Design: Systematic quantitative review of test accuracy studies.


Study Selection and Data Extraction: Two reviewers independently selected and extracted data on study characteristics, quality and accuracy. Accuracy data were used to form 2x2 contingency tables with spontaneous preterm birth before 34 and 37 weeks’ gestation and birth within 7-10 days of testing (only for symptomatic pregnant women) as reference standards.

Data Synthesis: Data were pooled to produce summary receiver operating characteristic (ROC) curves and summary likelihood ratios for positive (LR+) and negative (LR-) test results.

Results: There were 64 primary articles consisting of 28 accuracy studies in asymptomatic women and 40 accuracy studies in symptomatic women, including a total of 22,390 women. Among asymptomatic women, the best summary LR+ was 4.01 (95% confidence interval 2.93 to 5.49) for predicting birth before 34 weeks’ gestation, with corresponding summary LR- of 0.78 (0.72 to 0.84). Among symptomatic women, the best summary LR+ was 5.42 (4.36 to 6.74) for predicting birth within 7-10 days of testing, with corresponding summary LR- of 0.25 (0.20 to 0.31).

Conclusion: Cervico-vaginal fetal fibronectin test is most accurate in predicting spontaneous preterm birth within 7-10 days of testing among women symptomatic of threatened preterm birth before advanced cervical dilatation. These results enable clinicians to make a more rational approach to decision-making regarding in-patient admission, administration of antenatal steroids and in-utero transfer in such a situation.
Introduction

Spontaneous preterm birth occurs in 7-11% of pregnancies before 37 weeks' gestation\(^1,2\) and in 3-4% of pregnancies before 34 weeks' gestation.\(^3\) Majority of neonatal deaths of normally formed infants occurs when they are born before 34 weeks' gestation. Many of the surviving preterm infants, especially those from the earlier gestations, suffer serious morbidity such as broncho-pulmonary dysplasia, intraventricular haemorrhage, retrolental fibroplasia, neurodevelopmental problems and cognitive difficulties.\(^4,5\) Advances in perinatal healthcare have not altered the incidence of spontaneous preterm birth but effective management to reduce the associated complications exists. For example, the landmark Cochrane review\(^6\) (which is part of the Cochrane logo) showed that the use antenatal steroids significantly reduced morbidity and mortality. Timely institution of such therapy in clinical practice depends on accurate prediction of spontaneous preterm birth.

Many tests have been purported to be predictive of spontaneous preterm birth including cervico-vaginal fetal fibronectin testing. Fetal fibronectin is a glycoprotein found in amniotic fluid, placental tissue and the extracellular substance of the decidua basalis next to the placental intervillous space, and thought to be released into the cervico-vaginal milieu through mechanical or inflammatory-mediated damage to the membranes or placenta before birth.\(^7\) Swabs taken from either the ectocervix or posterior vaginal fornix, using an enzyme-linked immunosorbent assay containing FDC-6 monoclonal antibody, to detect fetal fibronectin\(^7\) may be used to predict spontaneous preterm birth.\(^8\) However, in clinical use, factors such as contamination of the sample with maternal blood\(^9\), sampling within 24 hours following intercourse\(^10\) and pre-eclampsia\(^11\) may reduce test accuracy and give false positives results.

In asymptomatic pregnant women, if fetal fibronectin could be used to identify a high-risk group, antenatal care may be optimised (e.g. by instituting closer antenatal surveillance) with view to see the pregnancy past 34 weeks' gestation, which is now an established milestone in perinatal outcome.\(^4,5\) On the other hand, if fetal fibronectin could predict imminent spontaneous preterm birth among women symptomatic of threatened spontaneous preterm birth, but before advance cervical dilatation, antenatal steroids, tocolytics and in-utero transfer (to optimise neonatal care) may be used accordingly. Antenatal steroids have maximal effectiveness among spontaneous preterm birth within 2-7 days after administration,\(^6\) and tocolytics are known to effectively delay birth for at least 2 days. Therefore, amongst symptomatic women, the main outcome of interest
was in predicting spontaneous preterm birth within 7-10 days of testing because this knowledge is likely to influence subsequent management.

Many primary studies claim that cervico-vaginal fetal fibronectin test is accurate in predicting spontaneous preterm birth in a clinical setting. However, these studies have not generally been conducted with large enough sample size to provide precise accuracy estimates. In addition, existing systematic reviews have been restricted to a few database searches, and their study selection has often been limited by language, and they have often ignored study quality assessment. These factors are known to introduce potential for bias. Against this background, we conducted a comprehensive and rigorous systematic review to obtain reliable estimates of fibronectin test accuracy. Our review focussed on two clinical groups of pregnant women, asymptomatic and symptomatic. We defined asymptomatic population as pregnant women without uterine tightenings/contractions, and symptomatic population as pregnant women with uterine tightenings/contractions and cervical dilatation of less than 2-3 cm.

**Methods**

Our review was carried out with a prospective protocol using widely recommended methodology.

Identification of studies

Our electronic searches targeted all diagnostic procedures among studies on prediction of spontaneous preterm birth. Studies were identified from various sources. We searched general bibliographic databases: MEDLINE (1966-2000), EMBASE (1980-2000), PASCAL (1973-2001) and BIOSIS (1969-2001). We also searched specialist computer databases: the Cochrane Library (2000:4), MEDION (1974-2000) (a database of diagnostic test reviews set up by Dutch and Belgian researchers), National Research Register (2000:4), SCISEARCH (1974-2001) and Conference Papers (1973-2000). The electronic search strategy used to develop the database is described in detail elsewhere. For completeness, we contacted individual experts with an interest in this field and the manufacturer of fetal fibronectin test to uncover grey literature. We also checked reference lists of known reviews and primary articles to identify cited articles not captured by electronic searches.
Study selection and data extraction procedures
The study selection criteria were i) asymptomatic or symptomatic population of pregnant women, ii) cervico-vaginal fetal fibronectin testing before 37 weeks’ gestation, iii) known gestation at spontaneous birth, and iv) observational cohort design. Studies were selected in a two-stage process. First, the electronic searches were scrutinised by two reviewers independently (HH and LMB) and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Second, final inclusion or exclusion decisions were made on examination of these manuscripts. In cases of duplicate publication, the most recent and complete versions were selected. There were no language restrictions but studies with case-control design were excluded. The assessment of English, French, Spanish language manuscripts was performed independently by two reviewers (HH and LMB), German language manuscripts by one reviewer (LMB) and other language manuscripts by people who had command of the language to allow data extraction from the manuscripts. Any disagreements about inclusion/exclusion were resolved by consensus or arbitration by a third reviewer (KSK).

Information was extracted from each selected article on study characteristics, quality and accuracy results. Study characteristics consisted of women’s risk classifications, test characteristics and reference standards of the test. In studies where multiple tests were performed, we considered any positive result in a serial testing as a positive result overall. Accuracy data were used to construct 2 x 2 tables of test result and spontaneous preterm birth, which served as the reference standard. Data were extracted from studies on asymptomatic and symptomatic women for information on spontaneous preterm birth before 34 and 37 weeks’ gestation. In addition, for symptomatic women, data on spontaneous preterm birth within 7-10 days of testing were also extracted. The data extraction form was piloted and tested for repeatability on the first eight manuscripts that we obtained. Overall, the observer agreement regarding the various components of data extraction form was 90-100%; with kappa values ranges from 0.9 to 1.0.

Methodological quality assessment
All manuscripts meeting the selection criteria were assessed for their methodological quality. We defined quality as the confidence that the study design, conduct and analysis minimised bias in the estimation of test accuracy. Current empirical evidence relates bias to items: case-control study designs, lack of blinding of carer to test results, non-consecutive patient enrolment, non-prospective data collection, inadequate test description, use of different reference tests, partial verification, lack of description of either the population or the reference test. The latter 4 items
Chapter 4

are, however, not relevant to our review because they refer to delivery of neonates (preterm or term births). Therefore, we considered a study to be of good quality if it used a prospective design, consecutive enrolment, adequate test description (to allow replication by others), and blinding of the test result from clinicians managing the patients.29

Data synthesis

Data were synthesised separately for studies on asymptomatic and symptomatic women with spontaneous preterm birth before 34 and 37 weeks' gestation. For symptomatic women, data were also synthesized for spontaneous preterm birth within 7-10 days of testing. Heterogeneity of diagnostic odds ratios was assessed graphically (using forest plots) and statistically (using chi-squared test) to aid in decisions on how to proceed with quantitative synthesis.30 For each outcome within the two populations, there was either graphical or statistically significant heterogeneity. Possible sources of heterogeneity were explored by meta-regression analysis using various independent explanatory variables defined a priori. These variables were: risk classifications (high or low as defined by the authors), multiple gestation (included or excluded), type of recruitment (consecutive or others), digital exam before testing (yes or no), sexual intercourse within 24 hours preceding testing (yes or no), bleeding before testing (yes or no), methods of testing (laboratory or bedside), serial testing (yes or no), gestation at testing for asymptomatic women (before or after 24 weeks), blinding of test results (yes or no), study design (prospective or retrospective), and publication language (English or other). Where a variable presence was not explicitly stated, it was treated as “no” in the meta-regression analysis. As our meta-regression analysis failed to explain the observed heterogeneity we proceeded with meta-analysis using random effects model.31 Consequently, the pooled results should be interpreted with caution. To aid in interpretation we examined the accuracy estimate of the highest quality studies included in our review.

Summary receiver operating characteristic (ROC) curves were used as measures of accuracy for all included studies regardless of their thresholds. Summary likelihood ratios (LR) were used as measures of accuracy for studies using 50 ng/ml as their thresholds. Area under summary ROC curve provided an average measure of accuracy from the combined studies (especially when there are different test thresholds) and a convenient way of comparing accuracy of the test for different outcomes.33 Summary LRs indicated by how much a given test result will raise or lower the probability of having spontaneous preterm birth. Using summary LRs, we determined post-test probabilities by Bayes’ theorem as follows: post-test probability = LR x pre-test probability/[1 -
Accuracy of cervicovaginal fetal fibronectin in predicting spontaneous preterm birth

pre-test probability x (1-LR)]. In this way, LRs are more clinically meaningful than sensitivities or specificities, for which meta-analysis are generally not recommended. To detect publication and related bias, we undertook funnel plot (diagnostic odds ratio vs. reciprocal of its standard error) analysis. All statistical analyses were performed using SPSS v. 10 and Stata 7.0 statistical packages.

Results
Literature identification and study quality
Figure 1 summarises the process of literature identification and selection. In total, 64 primary articles met the selection criteria. They consisted of 28 accuracy studies in asymptomatic women and 40 studies in symptomatic women, including a total of 22,390 women. Table 1 summarises each study's salient features according to whether the population was asymptomatic or symptomatic, and their risk classifications. The methodological quality is summarised in Figure 2. Thirteen (19%) studies, 7 among asymptomatic and 6 among symptomatic women, fulfilled all four criteria for good quality. All studies except three (which accounted for 0.28% of the total 22,390 women in our review), used thresholds of 50 ng/ml for an abnormal test result.
Chapter 4

Figure 1: Study selection process for systematic review of cervico-vaginal fetal fibronectin test.

Total citations identified from electronic searches to capture primary articles on all tests for predicting preterm birth: n= 30,061

Citations excluded after screening titles and/or abstracts: n = 29,972

Primary articles on cervico-vaginal fetal fibronectin test accuracy retrieved for detailed evaluation: n=116

from electronic search  n = 89
from reference lists n = 26
contact with manufacturer n =2 (from company’s website www.adeza.com)

Articles excluded with reasons:
Not test accuracy study: n=15
Duplicate publications or more complete data sets are available: n=9
Lack of data to construct 2x2 table: n=5
Lack of original data i.e. reviews, letter: n=22
Unobtainable: n=2
Total excluded n=53

Primary articles included in systematic review: n = 64 (see Table 1 for further details)
No. of studies included in these articles = 68*

Studies on asymptomatic women =28
Low risk = 8 studies
High risk = 9 studies
Risk not categorized = 11 studies

Studies on symptomatic women = 40
Low risk = 5 studies
High risk = 4 studies
Risk not categorized = 31 studies

Φ See Appendix 1 for complete reference list of excluded studies. *Total number of studies exceeded 64 because some primary articles provided data on more than one study.
Table 1: Characteristic of studies on test accuracy of cervico-vaginal fetal fibronectin in predicting spontaneous preterm birth

<table>
<thead>
<tr>
<th>Study</th>
<th>Language</th>
<th>Quality of study</th>
<th>Population</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
<th>Gestation Of Testing (weeks)</th>
<th>Type of Test &amp; Frequency</th>
<th>Swab site</th>
<th>Cut-off level (ng/ml)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic - High Risk</td>
<td>English</td>
<td>Blind</td>
<td>147</td>
<td>Twins</td>
<td>Cerclage, Placenta previa, Fetal anomaly</td>
<td>22-24, 26, 28, 30</td>
<td>Elina Multiple</td>
<td>Cervix</td>
<td>50</td>
<td>&lt;32 weeks</td>
</tr>
<tr>
<td>Goldberg, 1996</td>
<td></td>
<td>Consecutive Prospective</td>
<td>Test described</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morrison, 1996</td>
<td>English</td>
<td>Blind</td>
<td>85</td>
<td>Intact membranes, multiple gestations, or singlettons with previous history of spontaneous preterm birth, uterine anomaly, recurrent 2nd trimester miscarriage.</td>
<td>Cerclage, threatened preterm labor, vaginal bleeding, serious maternal and fetal condition</td>
<td>22-32</td>
<td>Elina Multiple</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;34 weeks</td>
</tr>
<tr>
<td>Tuling, 1996</td>
<td>English</td>
<td>Blind</td>
<td>68</td>
<td>Multiple gestation</td>
<td>None stated</td>
<td>24-36</td>
<td>Bedside Multiple Weekly</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Bitter, 1996</td>
<td>English</td>
<td>Consecutive Prospective</td>
<td>Test described</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobatin, 1996</td>
<td>English</td>
<td>Blind</td>
<td>102</td>
<td>Singletons pregnancies</td>
<td>Vaginal bleeding, Cortox &gt;24 hrs.</td>
<td>24-34</td>
<td>Bebe Elna* Multiple</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Nagazone, 1996</td>
<td>English</td>
<td>Blind</td>
<td>40</td>
<td>Intact membranes, previous history of spontaneous preterm birth or current multiple gestation.</td>
<td>None stated</td>
<td>24-34</td>
<td>Elina Multiple</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Nagonzoe, 1996</td>
<td>English</td>
<td>Blind</td>
<td>87</td>
<td>Intact membranes, multiple gestation.</td>
<td>Candida infections</td>
<td>24-34</td>
<td>Elina Multiple</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Olivera, 1998</td>
<td>English</td>
<td>Blind</td>
<td>52</td>
<td>Twins, Intact membranes, cervix and cervical length &gt;1cm.</td>
<td>Vaginal bleeding, Gastrosopic delivery. Disagreement between two observers in the results of the test.</td>
<td>24-34</td>
<td>Bedside Multiple</td>
<td>Not stated</td>
<td>50</td>
<td>&lt;34 weeks</td>
</tr>
<tr>
<td>Knotts, 1995</td>
<td>English</td>
<td>Blind</td>
<td>26</td>
<td>Previous history of spontaneous preterm births, preterm labor, preterm premature rupture of membranes, and stillbirth.</td>
<td>None stated</td>
<td>&gt;24</td>
<td>Elina Multiple</td>
<td>Cervix</td>
<td>50</td>
<td>&lt;36 weeks</td>
</tr>
<tr>
<td>Nagazone, 1995</td>
<td>English</td>
<td>Test described</td>
<td>102</td>
<td>Multiple gestation.</td>
<td>None stated</td>
<td>&gt;20</td>
<td>Elina Multiple</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;34 weeks</td>
</tr>
<tr>
<td>Asymptomatic - Low Risk</td>
<td>English</td>
<td>Blind</td>
<td>155</td>
<td>All asymptomatic women in unental clinic with known gestation.</td>
<td>Vaginal bleeding</td>
<td>24-33</td>
<td>Elina Single</td>
<td>Endocorvis</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Fauer, 1997</td>
<td></td>
<td>Consecutive Prospective</td>
<td>Test described</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holleman, 1995</td>
<td>English</td>
<td>Blind</td>
<td>133</td>
<td>Low risk singleton pregnancies, intact membranes.</td>
<td>Placenta previa, Vaginal bleeding, Cervical dilatation &gt;3cm or cervical efface, Threatened preterm labor &gt;26 weeks, Unknown date.</td>
<td>26-36</td>
<td>Elina Multiple</td>
<td>Exucorvis</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Chung, 1997</td>
<td>English</td>
<td>Blind</td>
<td>234</td>
<td>Singletons pregnancies without previous history of spontaneous preterm labor or birth, intact membranes.</td>
<td>Vaginal bleeding, Pre-eclampsia. Uncessed date.</td>
<td>28</td>
<td>Elina Single</td>
<td>Forex</td>
<td>50</td>
<td>&lt;34 weeks</td>
</tr>
</tbody>
</table>

Test described.
<table>
<thead>
<tr>
<th>Study</th>
<th>Language</th>
<th>Study Type</th>
<th>Sample Size</th>
<th>Intervention</th>
<th>Duration</th>
<th>Follow-up</th>
<th>Outcome</th>
<th>Route</th>
<th>Dose</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia 1999</td>
<td>Spanish</td>
<td>Blind Prospective Test described</td>
<td>263</td>
<td>Low risk singleton intact membrane</td>
<td>Collagen</td>
<td>24-37</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;32 weeks</td>
</tr>
<tr>
<td>Groenvangen 1996</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>108</td>
<td>Low risk singleton pregnancies intact membrane</td>
<td>Previous history of spontaneous preterm labor or birth</td>
<td>Vaginal Bleeding, Fetal anemia</td>
<td>Elisa Multiple 2 weekly</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Di Stefano 1999</td>
<td>English</td>
<td>Prospective Test described</td>
<td>60</td>
<td>Singleton pregnancies intact membrane</td>
<td>Previous history of spontaneous preterm labor or birth</td>
<td>Vaginal Bleeding, Fetal anemia, Cervical cerclage, Gestational age, Maternal or fetal complications during pregnancy</td>
<td>Elisa Multiple 2 weekly</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
</tr>
<tr>
<td>Goldenberg 1997</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>140</td>
<td>Singleton pregnancies intact membrane</td>
<td>Collagen, Fetal aneuromies or death, Vaginal bleeding, Recently treated bacterial vaginosis</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
<td></td>
</tr>
<tr>
<td>Zanoli 2000</td>
<td>Spanish</td>
<td>Blind Test described</td>
<td>30</td>
<td>Low risk primigravida</td>
<td>Collagen 24 hours, Recent usage of vaginal pessary</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>1000</td>
<td>&lt;34 weeks</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic 1, Risk Unclassified Cervix 1996</td>
<td>English</td>
<td>Blind Consecutive Prospective Test described</td>
<td>5166</td>
<td>Singleton pregnancies intact membrane</td>
<td>Fetal abnormalities</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;33 weeks</td>
<td></td>
</tr>
<tr>
<td>Heath 2000</td>
<td>English</td>
<td>Blind Consecutive Prospective Test described</td>
<td>438</td>
<td>Singleton pregnancies without medical or obstetrical complications</td>
<td>None stated</td>
<td>Elisa Single Not stated</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;34 weeks</td>
<td></td>
</tr>
<tr>
<td>Amoroso 1999</td>
<td>English</td>
<td>Consecutive Prospective Test described</td>
<td>2529</td>
<td>Singleton pregnancies</td>
<td>Placenta previa, Fetal anomalies</td>
<td>Elisa Multiple 2 weekly</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;34 weeks</td>
<td></td>
</tr>
<tr>
<td>Goldemberg 1996</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>1870</td>
<td>Singleton pregnancies of women who are not randomized to treatment for Tobsenosis vaginitis or Bacterial vaginitis</td>
<td>None stated</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;35 weeks</td>
<td></td>
</tr>
<tr>
<td>Goldemberg 2000</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>6508</td>
<td>Singleton pregnancies</td>
<td>None stated</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;38 weeks</td>
<td></td>
</tr>
<tr>
<td>Hix 1993</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>54</td>
<td>Intact membrane and amniotic cervix</td>
<td>Candida infection, Total amniocentesis, vaginal bleeding, placenta previa, and threatened preterm labor</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
<td></td>
</tr>
<tr>
<td>Ingol 1994</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>75</td>
<td>Intact membrane</td>
<td>Fetal anomalies, Placenta previa, genital or urinary infection, use of antibiotics in the preceding 7 days</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
<td></td>
</tr>
<tr>
<td>Lockwood 1993</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>429</td>
<td>Singleton pregnancies of women with an amnion or intact cervix</td>
<td>None stated</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;35 weeks</td>
<td></td>
</tr>
<tr>
<td>Goldemberg 1997</td>
<td>English</td>
<td>Blind Prospective Test described</td>
<td>2929</td>
<td>Singleton pregnancies</td>
<td>Placenta previa, Fetal anomalies</td>
<td>Elisa Multiple 2 weekly</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
<td></td>
</tr>
<tr>
<td>Vermaas 1996</td>
<td>French</td>
<td>Test described</td>
<td>58</td>
<td>None stated</td>
<td>Collagen 24 hours and vaginal bleeding</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
<td></td>
</tr>
<tr>
<td>Symptomatic High Risk Malagana 1995</td>
<td>French</td>
<td>Blind Prospective Test described</td>
<td>185</td>
<td>Intact membranes, cervix, cervical changes, amniotic membrane, collagen</td>
<td>Psychiatric condition, collagen 24 hours</td>
<td>Elisa Single</td>
<td>Ferris</td>
<td>50</td>
<td>&lt;37 weeks</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Language</td>
<td>Year</td>
<td>Study Type</td>
<td>Test</td>
<td>Week(s)</td>
<td>Condition</td>
<td>Drug</td>
<td>Dose</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>------</td>
<td>------------</td>
<td>------</td>
<td>---------</td>
<td>----------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Sarbaj 1997</td>
<td>German</td>
<td>Blind</td>
<td>Prospective</td>
<td>Test described</td>
<td>56</td>
<td>Multiple gestation, uterine malformation, congenital infection, vaginal bleeding, previous history of spontaneous or preterm birth.</td>
<td>Preterm premature rupture of membranes, Placenta previa. Unknown cause.</td>
<td>Single</td>
<td>24-32</td>
<td>Elisa</td>
</tr>
<tr>
<td>Symptomatic: Low Risk</td>
<td>English</td>
<td>Blind</td>
<td>Prospective</td>
<td>Test described</td>
<td>112</td>
<td>Intact membrane, cervical dilation &lt;2 cm.</td>
<td>None stated</td>
<td>Single</td>
<td>22-35</td>
<td>Elisa</td>
</tr>
<tr>
<td>Banerji 1997</td>
<td>English</td>
<td>Blind</td>
<td>Test described</td>
<td>124</td>
<td>Singletons and twin pregnancies. Intact membrane, cervical dilation &lt;3 cm.</td>
<td>Vaginal bleeding.</td>
<td>Cervix &lt; 24 hours.</td>
<td>Bedside</td>
<td>Single</td>
<td>Eustaccin</td>
</tr>
<tr>
<td>Ciklo 1995</td>
<td>Czech</td>
<td>Prospective</td>
<td>Test described</td>
<td>84</td>
<td>Intact membrane.</td>
<td>None stated</td>
<td>Single</td>
<td>24-34</td>
<td>Bedside</td>
<td>Furinix</td>
</tr>
<tr>
<td>Gubbargh 1995</td>
<td>English</td>
<td>Blind</td>
<td>Test described</td>
<td>11</td>
<td>Symptomatic women in their first pregnancies. Intact membrane.</td>
<td>None stated</td>
<td>Single</td>
<td>28-36</td>
<td>Elisa</td>
<td>Eustaccin</td>
</tr>
<tr>
<td>Haugel 1994</td>
<td>German</td>
<td>Prospective</td>
<td>Test described</td>
<td>34</td>
<td>Intact membrane.</td>
<td>Fetal anomaly and cervical cerclage.</td>
<td>Single</td>
<td>24-36</td>
<td>Elisa</td>
<td>Furinix</td>
</tr>
<tr>
<td>Author</td>
<td>Year</td>
<td>Language</td>
<td>Study Design</td>
<td>Score</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>----------</td>
<td>--------------</td>
<td>-------</td>
<td>------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laken</td>
<td>1997</td>
<td>English</td>
<td>Blind</td>
<td>763</td>
<td>Intact membrane. Cervical dilation (&lt;) 2 cm.</td>
<td>None stated.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McKenna</td>
<td>1996</td>
<td>English</td>
<td>Consecutive</td>
<td>50</td>
<td>Cervical dilation (&lt;) 3 cm.</td>
<td>Cervix = 24 hours. Critical medical or surgical condition.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Duration  | 25-35 | &quot;Bedside Single&quot; | Fonnix | 50 | &lt;7 days |
| Duration  | 25-34 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 23-34 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 24-34 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 24-36 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 24-35 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 24-36 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 24-36 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 25-35 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 26-34 | &quot;Elisa Single&quot; | Fonnix | 50 | &lt;3 weeks |
| Duration  | 28-36 | &quot;Elisa Single&quot; | Cervix | 250 | &lt;3 weeks |
| Duration  | 20-34 | &quot;Bedside Single&quot; | Fonnix | 50 | &lt;3 weeks |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Language</th>
<th>Type of Study</th>
<th>Number of Participants</th>
<th>Criteria</th>
<th>Cause of Delivery</th>
<th>Postpartum Follow-up</th>
<th>Duration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pecorino 1991^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>125</td>
<td>Singleton, twin pregnancies and 1 gestation, intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Placenta previa, abruptio placentae, trauma leading to preterm labor.</td>
<td>Elisa single</td>
<td>&lt;7 days</td>
<td>37 weeks</td>
</tr>
<tr>
<td>Rizzo 1986^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>108</td>
<td>Singleton pregnancies, intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Fetal or maternal complications.</td>
<td>Elisa single</td>
<td>&lt;37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Rizzo 1997^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>106</td>
<td>Singleton pregnancies, intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Fetal or maternal complications.</td>
<td>Bedside single</td>
<td>&lt;37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Rosenburg 1997^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>76</td>
<td>Singleton pregnancies, intact membrane, cervical dilatation &lt; 2 cm.</td>
<td>Gestation &gt; 24 or &lt; 34 weeks.</td>
<td>Elisa single</td>
<td>&lt;37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Wennempergen 1997^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>101</td>
<td>Twin pregnancies, intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Coitus &lt; 24 hours, vaginal bleeding, abruptio placentae, cervical dilatation &gt; 3 cm.</td>
<td>Elisa multiple 2 weekly</td>
<td>&lt;34 weeks</td>
<td>50 Forix</td>
</tr>
<tr>
<td>Chaljub 1999^*</td>
<td>English</td>
<td>Retrospective, Test described</td>
<td>50</td>
<td>Singleton pregnancies, intact membrane, cervical dilatation &lt; 2 cm.</td>
<td>Placenta previa, abruptio placentae, cervical dilatation &gt; 3 cm.</td>
<td>Bedside single</td>
<td>&lt;34 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Gibson 2000^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>150</td>
<td>Intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Fetal abnormalities, severe uterine hypertonus, acute or chronic diseases, diabetes, placenta previa, abruptio placentae, cervical dilatation &gt; 3 cm.</td>
<td>Bedside single</td>
<td>&lt;7 days</td>
<td>36 weeks</td>
</tr>
<tr>
<td>Vair 1996^*</td>
<td>English</td>
<td>Prospective, Test described</td>
<td>46</td>
<td>Intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>None noted.</td>
<td>Bedside single</td>
<td>&lt;37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Cao 1995^*</td>
<td>English</td>
<td>Test described</td>
<td>175</td>
<td>Intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>None noted.</td>
<td>Elisa single</td>
<td>50 Forix</td>
<td></td>
</tr>
<tr>
<td>Manusam 1996^*</td>
<td>French</td>
<td>Retrospective, Test described</td>
<td>90</td>
<td>Intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Vaginal bleeding, coitus &lt; 24 hours.</td>
<td>Elisa single</td>
<td>37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Ramel 1997^*</td>
<td>French</td>
<td>Test described</td>
<td>73</td>
<td>Symptomatic women with cervical changes and requiring surgery.</td>
<td>None noted.</td>
<td>Elisa single</td>
<td>37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Veronese 1996^*</td>
<td>French</td>
<td>Test described</td>
<td>86</td>
<td>Singleton and twin pregnancies, cervical dilatation &lt; 3 cm.</td>
<td>Coitus &lt; 24 hours, vaginal bleeding, cervical dilatation &lt; 3 cm.</td>
<td>Elisa single</td>
<td>37 weeks</td>
<td>Forix 50</td>
</tr>
<tr>
<td>Lopez 2000^*</td>
<td>English</td>
<td>Retrospective, Test described</td>
<td>85</td>
<td>Singleton pregnancies, intact membrane, cervical dilatation &lt; 3 cm.</td>
<td>Unexplained death, lost to follow up, incomplete data.</td>
<td>Elisa single &amp; multiple</td>
<td>24-35 days</td>
<td>Not stated</td>
</tr>
</tbody>
</table>

* Elisa = Enzyme-linked immunosorbent assay
^ If quality item (i.e., clinician blinded to the result, consecutive population enrollments, prospective or retrospective study design or description of the test) is not stated, it was either not reported or was not explicitly clear.
^* Asymptomatic population defined as pregnant women without uterine tightening or contractions.
^** Symptomatic population defined as pregnant women with uterine tightening or contractions but without cervical dilatation of more than 2-3 cm.
^† Risk classifications, if stated, were based on either clinical or historical factors as defined by the study's author(s), e.g. history of previous spontaneous preterm birth, history of recurrent miscarriages, multiple gestations, etc.
Chapter 4

Fibronectin test in asymptomatic women

The accuracy of the test was studied using bedside (n=3) or laboratory (n=26) methods, either on a single occasion (n=13) or by serial testing (n=16) at various gestations (Table 1). In these studies, fibronectin was used as a screening tool in low risk pregnancy (n=8) or as a selective screening tool in high-risk pregnancy (n=9) (Table 1). Most studies were carried out during the second or early third trimester of the pregnancy. Meta-regression analysis showed that test accuracy was not dependent on method of testing, serial testing, risk classifications or gestation at testing. Cervico-vaginal fetal fibronectin test showed different accuracy estimates in predicting spontaneous preterm birth for the various gestations of interest as shown in Figure 3 and Figure 4. The effect of these on changes in probabilities of spontaneous preterm birth is shown in Figure 4. exploration of study quality as a source of heterogeneity did not reveal statistically significant differences in accuracy estimates of studies with high and low quality features. The accuracy estimates of studies that fulfilled all four of the quality criteria were generally consistent with the pooled results. For example, the median likelihood ratios for predicting spontaneous preterm birth before 34 weeks’ gestation among the five highest quality studies were 3.99 (inter-quartile range 1.73 to 10.18) for a positive test result and 0.38 (inter-quartile range 0.10 to 0.69) for a negative test result.

Figure 2: Methodological quality of studies included in the systematic review. Data presented as 100% stacked bars. Figures in the stacks represent number of studies.
Accuracy of cervicovaginal fetal fibronectin in predicting spontaneous preterm birth

Fibronectin test in symptomatic women

The accuracy of the test was studied using bedside (n=11) or laboratory (n=30) methods, either on a single occasion (n=35) or serially (n=5) (Table 1). Meta-regression analysis showed that test accuracy was not dependent on method of testing, serial testing, or risk classifications. As with fetal fibronectin test in asymptomatic women, the test showed different accuracy estimates in predicting spontaneous preterm birth for the various gestation of interest shown in figure 3 and figure 4. The effect of these on changes in probabilities of spontaneous preterm birth is shown in Figure 4. Exploration of study quality as a source of heterogeneity did not reveal statistically significant differences in accuracy estimates of studies with high and low quality features. The accuracy estimates of studies that fulfilled all four of the quality criteria were generally consistent with the pooled results. For example, the median likelihood ratios for predicting for predicting spontaneous preterm birth within 7-10 days of testing among the four highest quality studies were 6.16 (inter-quartile range 4.53 to 7.33) for a positive test result and 0.32 (inter-quartile range 0.01 to 0.45) for a negative test result. Funnel plot analysis did not show any evidence of asymmetry to indicate presence of publication or related bias for the main outcomes.

Figure 3: Summary Receiver Operating Characteristic (ROC) curves and areas (95% CI) for cervicovaginal fetal fibronectin test in predicting spontaneous preterm birth in asymptomatic and symptomatic women.
Figure 4: Pooled estimates of likelihood ratios (LRs) for cervico-vaginal fetal fibronectin test and their impact on predictive probabilities of spontaneous preterm birth in asymptomatic and symptomatic women.

<table>
<thead>
<tr>
<th>Population/ Birth Outcome (no. of studies)</th>
<th>Weighted Pre-test Probability (Median %)</th>
<th>Summary Likelihood ratios (LRs) (95% CI)</th>
<th>Post-test Probabilities (% (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Negative</td>
<td>Test Positive</td>
<td>Test Negative</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td></td>
<td></td>
<td>3.0 (2.6-3.2)</td>
</tr>
<tr>
<td>&lt;34 weeks (n=12)</td>
<td>4.2</td>
<td></td>
<td>13.7 (10.4-17.8)</td>
</tr>
<tr>
<td>&gt;37 weeks (n=23)</td>
<td>0.78 (0.72-0.84)</td>
<td>4.01 (2.95-5.49)</td>
<td>0.78 (0.72-0.84)</td>
</tr>
<tr>
<td></td>
<td>0.52 (0.44-0.62)</td>
<td>2.94 (2.47-3.50)</td>
<td>0.52 (0.44-0.62)</td>
</tr>
<tr>
<td>Symptomatic women</td>
<td></td>
<td></td>
<td>3.0 (2.6-3.2)</td>
</tr>
<tr>
<td>7-14 days (n=14)</td>
<td>3.0</td>
<td></td>
<td>14.6 (11.9-17.2)</td>
</tr>
<tr>
<td>&lt;54 weeks (n=9)</td>
<td>0.25 (0.20-0.31)</td>
<td>5.42 (4.36-6.76)</td>
<td>0.25 (0.20-0.31)</td>
</tr>
<tr>
<td>&gt;37 weeks (n=57)</td>
<td>15.7</td>
<td>3.64 (2.32-5.73)</td>
<td>15.7 (10.9-21.6)</td>
</tr>
<tr>
<td></td>
<td>0.40 (0.41-0.56)</td>
<td>3.27 (2.74-3.92)</td>
<td>0.40 (0.41-0.56)</td>
</tr>
</tbody>
</table>

Discussion
Our results show that the cervico-vaginal fetal fibronectin test exhibits a range of accuracy in predicting various spontaneous preterm birth outcomes. The test is most accurate in predicting spontaneous preterm birth within 7-10 days of testing among women symptomatic of threatened preterm birth before advanced cervical dilatation.

Quality of our review
The strength of our inferences depends on the rigour of our methodology. In contrast to the previous four systematic reviews,12-13 we have identified 64 studies (at least twice as many studies as the largest previous review14) because we did not limit our search to a single database13,15 nor did we apply language restrictions.13 Cognisant that meta-analysis of test accuracy studies are fraught with difficulty due to poor methodological quality of the primary studies, we scrutinised the selected studies for their quality, an assessment undertaken in only one previous review.15 Methodological issues that may overestimate accuracy such as case-control design, absence of test descriptions, and different reference tests,28 were not applicable to the studies we reviewed. Our quality assessment, however, were affected by poverty of reporting in some instances. Moreover, quality was not a significant factor explaining differences between their results. Assessment of heterogeneity and exploration for reasons behind heterogeneity were planned a priori. In the presence of unexplained heterogeneity, we proceeded with caution, pooling data with random effects model, which produces a
wider confidence interval.\textsuperscript{16} However, due to the large number of studies in our review the accuracy estimates were generally more precise compared to previous reviews.

Clinical application
The clinical impact of the accuracy estimates we produced depends on how the resultant changes in probabilities due to fibronectin testing alter therapeutic effectiveness in decision-making.\textsuperscript{55} We demonstrate this impact with an example of decision making about the use of antenatal steroids\textsuperscript{6} in symptomatic pregnant women with threatened preterm birth at 31 weeks' gestation as shown in Table 2. The absolute effect of antenatal steroids depends on the risk of spontaneous preterm birth following presentation. The higher the risk, the lower the number of patients needed to be treated (NNT) to prevent one case of RDS and vice versa. The risk, and hence the therapeutic benefits, depends not only on the gestational age at presentation, but also on the post-test probabilities of spontaneous preterm birth associated with fibronectin testing. As shown in Table 2, if steroids were to be used for all symptomatic women at this gestation without fibronectin testing then 109 women would need to be treated with antenatal steroids to prevent 1 case of RDS. For a positive test result, the NNT would be 20, a figure considerably lower than that without testing. On the other hand, for a negative test result, the NNT would be 509.
### Table 2: Cervico-vaginal fetal fibronectin testing among symptomatic women and the Number of women Needed to be Treated (NNT) at 31 weeks’ gestation with antenatal steroids to prevent one case of neonatal respiratory distress syndrome (RDS) associated with spontaneous preterm birth within 7-10 days of testing.

<table>
<thead>
<tr>
<th>Fibronectin test result at 31 weeks’ gestation</th>
<th>Probability of spontaneous preterm birth within 7-10 days of testing (%)</th>
<th>Risk of RDS at 32 weeks’ gestation&lt;sup&gt;6,57&lt;/sup&gt;</th>
<th>Rate of RDS&lt;sup&gt;5&lt;/sup&gt; at 32 weeks’ gestation (%)</th>
<th>NNT&lt;sup&gt;66&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No testing</td>
<td>4.5&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.53</td>
<td>2.0</td>
<td>109</td>
</tr>
<tr>
<td>Test positive</td>
<td>20.6&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.53</td>
<td>11.0</td>
<td>20</td>
</tr>
<tr>
<td>Test negative</td>
<td>1.0&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.53</td>
<td>0.4</td>
<td>509</td>
</tr>
</tbody>
</table>

* Pre-test probability of spontaneous preterm birth within 7-10 days of testing for symptomatic pregnant women presenting at 31 weeks’ gestation.<sup>21,22,24,39,51,56-65</sup>

* Information on the calculation of probabilities using likelihood ratios shown in Figure 4:
- Pre-test probability of spontaneous preterm birth within 7-10 days (i.e. no testing) for women presenting at 31 weeks’ gestation = 4.5%
- This value is converted to pre-test odds = 4.5/(100-4.5) = 0.047
- Post-test odds for spontaneous preterm birth among women with a positive test = pre-test odds x LR+ = 0.047 x 5.45= 0.26
- This is then converted to post-test probability = 0.26/(0.26+1) = 0.206 = 20.6%
  (A similar calculation may be carried out for the negative test result using LR- found in Figure 4)

* The rate of RDS is calculated as follows:
- Probability of spontaneous preterm birth for a positive fibronectin test result at 32 weeks (31 weeks + 7-10 days) = 20.6%
- The risk of RDS at this gestation = 0.53<sup>6,57</sup>
- Therefore, the probability of a neonate suffering from RDS in woman who tested positive = 20.6 x 0.53 = 11%.
  (A similar calculation may be carried out for the negative test result)

### An example calculation of NNT to prevent 1 case of RDS among women who tested positive:
- Rate of RDS at 32 weeks’ gestation = 11%
- This value is converted to odds of RDS without treatment = 11/(100-11) = 0.12
- Odds of treatment benefit = 0.12 x 0.53 = 0.064 (where 0.53 = odds ratio [OR] for treatment benefit of antenatal steroids, obtained from Cochrane review<sup>6</sup>, which coincidentally, is the same figure as the risk for RDS at 32 weeks’ gestation)
- This is then converted to the rate of RDS following antenatal steroid treatment = 0.064/(1+0.064) = 0.059
- Rate difference of RDS between treatment and without antenatal steroid treatment = 0.11-0.059 = 0.051
- NNT = 1/0.051 = 20
- This means that with a positive fibronectin test, 17 symptomatic pregnant women who presented at 31 weeks’ gestation need to be treated with antenatal steroids to prevent 1 case of RDS
  (A similar calculation may be carried out for the negative test result)

This approach will allow clinicians to make explicit decisions based on more realistic probabilities generated by fibronectin testing and provides a framework for the use of diagnostic evidence in therapeutic decision-making. Specifically, our results enable clinicians to make a more rational approach to decision-making regarding in-patient admission, administration of antenatal steroids and in-utero transfer in symptomatic women presenting with threatened spontaneous preterm birth. Future research should focus on undertaking high quality primary studies of test accuracy to improve our ability to predict spontaneous preterm birth.
Acknowledgements
This project was funded by Wellbeing grant no. K2/00. The authors would like to thank Fujian Song, Malgorzata Adamecyzck and Pavlina Jungova for their help in extracting relevant data from Chinese, Polish and Czech’s manuscripts respectively. The authors would also like to thank NHS Centre for Reviews and Dissemination at York for their contribution to the database searches.

References


Accuracy of cervicovaginal fetal fibronectin in predicting spontaneous preterm birth


Accuracy of cervicovaginal fetal fibronectin in predicting spontaneous preterm birth


Appendix 1: Reference list of excluded studies. Not test accuracy study:


Duplicate publications or more complete data sets are available:


Lack of data to construct 2x2 table:


31. Lack of original data (e.g. reviews, letter, editorial, etc):


Accuracy of cervicovaginal fetal fibronectin in predicting spontaneous preterm birth


Unobtainable:

