RAPID DIAGNOSTICS

Prospective, multi-centre clinic-based evaluation of four rapid diagnostic tests for syphilis

D Mabey, R W Peeling, R Ballard, A S Benzaken, E Galbán, J Changalucha, D Everett, R Balira, D Fitzgerald, P Joseph, S Nerette, J Li, H Zheng

Sex Transm Infect 2006;**82**(Suppl V):v13–v16. doi: 10.1136/sti.2006.022467

See end of article for authors' affiliations

Correspondence to: Professor David Mabey, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK; david.mabey@lshtm.ac.uk

Accepted 15 September 2006

Objectives: To evaluate prospectively four rapid, point-of-care serological tests for syphilis in prenatal or high risk populations in four countries.
Methods: Tests were performed on consecutive clinic attenders, using whole blood in the clinic, and whole blood and serum in the laboratory. The sensitivity and specificity of each test was evaluated, using a standard treponemal test (*Treponema pallidum* haemagglutination assay (TPHA) or fluorescent treponemal antibody, absorbed (FTA-ABS) as gold standard. Non-treponemal tests (rapid plasma reagin (RPR) or venereal diseases research laboratory (VDRL) tests) were also performed on all subjects at three sites.
Results: The specificity of each rapid test was >95% at each site. Sensitivities varied from 64–100% and, in most cases, were lower when whole blood was used rather than serum.
Conclusions: Rapid serological tests for syphilis are an acceptable alternative to conventional laboratory tests. Since they do not require equipment or electricity, they could increase coverage of syphilis screening, and enable treatment to be given at the first clinic visit.

The incidence of syphilis is increasing in many parts of the world, including China and Eastern Europe.[1 2] Even in regions where it has long been considered under control, such as Western Europe, its incidence has increased in recent years among high risk groups such as men who have sex with men.[3] In many developing countries syphilis remains a major cause of adverse pregnancy outcome.[4–7] A recent study in Tanzania found that it was responsible for some 50% of all stillbirths.[8] Screening and treatment of pregnant women for syphilis remains cost-effective even when the prevalence is low.[9] In Tanzania, where the prevalence of syphilis in pregnant women was found to be approximately 8%, it is among the most cost-effective health interventions available, at less than US$11 per disability-adjusted life year (DALY) saved.[10]

In almost all countries it is official health policy to offer screening for syphilis to all pregnant women. The reality is, unfortunately, rather different. It has been estimated that less than 30% of pregnant women are screened for syphilis in sub-Saharan Africa.[4 11] A study in Bolivia showed that, although 76% of the study population received antenatal care, only 17% were screened for syphilis during pregnancy.[7] A major barrier to antenatal syphilis screening is that the non-treponemal tests generally used (rapid plasma reagin (RPR) or venereal diseases research laboratory (VDRL) tests) require a laboratory with trained personnel, refrigeration for storage of reagents, and electricity to run the refrigerator, centrifuge to separate serum, and shaker to perform the test. The refrigeration requirement also affects consistent availability of reagents because of difficulties with procurement and storage.

Since access to laboratory facilities is generally not possible in remote areas in developing countries, blood or serum samples must be transported to regional or central facilities for testing. Results are therefore often available only days or weeks later. Studies have shown that, even when this simple policy is followed, only a small proportion of infected women receive treatment when RPR testing is performed off site, since many do not return for their results, and specimens and results are lost in transit.[12] A number of demonstration projects have shown that decentralisation of syphilis screening followed by immediate, same-day treatment can be effective in reducing perinatal mortality.[13–17]

However, even in areas where services are available, there are technical difficulties associated with performing serological tests at primary health care settings. In particular, maintaining trained personnel and assuring quality standards and supplies of tests and treatment are problematic.[18 19] Simple, point-of-care treponemal tests provide an important opportunity to improve coverage. Recent evaluations have shown that a number of simple, point-of-care tests for syphilis, in a dipstick or cassette format, compare favourably with the standard treponemal tests.[20–23] The advantage of these tests is that, unlike RPR or VDRL, they can be stored at room temperature in any health facility, do not require any equipment and can use whole blood obtained by finger pricks.

We have prospectively evaluated the performance of four of these tests, previously shown to perform adequately using stored sera,[24] in four countries in which syphilis is a major public health problem. We have measured their sensitivity and specificity using whole blood tested in the clinic, and serum and whole blood tested in the laboratory.

METHODS

We evaluated four rapid tests: Determine Syphilis TP (Abbott Laboratories, Tokyo, Japan); VisiTect Syphilis (Omega Diagnostics, Alloa, UK); Syphicheck-WB (Qualpro Diagnostics, Goa, India); and SD Bioline Syphilis 3.0 (Standard Diagnostics, Kyonggi-do, Korea). All had been shown to perform adequately against a *Treponema pallidum* haemagglutination assay (TPHA) or *Treponema pallidum* particle agglutination assay (TPPA) gold standard on stored serum samples,[24] and in each case the manufacturer stated that the tests could be performed on either whole blood or serum.

Abbreviations: DALY, disability-adjusted life year; EDTA, ethylene diamine tetraacetic acid; FTA-ABS, fluorescent treponemal antibody, absorbed; RPR, rapid plasma reagin; STI, sexually transmitted infections; TPHA, *Treponema pallidum* haemagglutination assay; TPPA, *Treponema pallidum* particle agglutination assay; VDRL, venereal diseases research laboratory

The tests were evaluated prospectively at four sites, one each in Brazil, China, Haiti and Tanzania. Each site evaluated two rapid tests in parallel at both the clinic and the laboratory against a reference standard test of either TPPA, TPHA or fluorescent treponemal antibody, absorbed (FTA-ABS). These widely used treponemal tests were considered appropriate since the rapid tests under evaluation were also treponema-specific, rather than reagin type tests. When 50 positive reference tests had been obtained, the site switched to evaluating the remaining two rapid tests.

Study subjects

Consecutive patients were recruited in clinics serving populations at moderate to high risk of syphilis. Patients aged less than 18 years and those with a past history of syphilis and known positive serology were excluded. After giving informed consent, patients were interviewed and examined according to routine clinic protocol at the site. Treatment was given to those with positive RPR or VDRL results.

In Haiti, consecutive new patients were recruited at a free HIV voluntary counselling and testing centre and sexually transmitted infections (STI) clinic in Port au Prince between January 2003 and March 2004. In China, consecutive study subjects were recruited at the STI diagnosis and treatment centre of Peking Union Medical College Hospital between September 2003 and April 2004. In Tanzania, consecutive pregnant women were recruited at the main government antenatal clinic in Mwanza between September 2003 and June 2004. In Brazil, consecutive patients presenting to the STI clinic of the Fundação Alfredo da Matta in Manaus were recruited between March 2003 and May 2004.

Laboratory methods

Except in Brazil, 10 ml of blood was collected from each patient, of which 5 ml was placed in an EDTA tube for whole blood testing, and 5 ml in plain tubes for serum. In Brazil, a finger prick sample was collected for the whole blood test. An aliquot of whole blood was used immediately to perform the rapid tests according to the manufacturers' instructions at the clinic. To determine variability in the interpretation of test results, each result was read independently by two clinic staff members. The remainder of the blood was transported to the laboratory where trained laboratory personnel performed the rapid tests with both whole blood and serum (except in Brazil, where only serum was tested in the laboratory). The serum sample was used for reference testing. Laboratory staff were not aware of the rapid test results obtained at the clinic.

The reference standard test for syphilis in Haiti was TPHA (Human, Wiesbaden Germany); in China was TPHA (Omega Diagnostics, UK); in Tanzania was TPPA (Fujirebio, Tokyo, Japan); and in Brazil was FTA-ABS (WAMA Diagnostica, São Paulo, Brazil). Each test was performed by laboratory staff on sera according to manufacturer's directions. RPR testing was performed on all sera in Haiti and Tanzania, and titres were determined on positive samples. In Haiti the RPR reagent was obtained from Human and in Tanzania from Omega Diagnostics. In Brazil the VDRL was used (Wiener Laboratories, Rosario, Argentina), and titres determined.

Ethical approval

In addition to being approved by the World Health Organization ethics committee, the study was approved by the National Ethical Committee in Brasília; the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania; the GHESKIO Center's institutional review board in Haiti and the Cornell University institutional review board in New York; and the ethics committee of Peking Union Medical College.

Data analysis

The sensitivity and specificity of each rapid test against the gold standard were calculated according to standard methods. Ninety-five per cent confidence intervals (95% CI) were calculated according to the formula $1.96\ \sqrt{p(1-p)/n}$, where p is the point estimate of sensitivity (or specificity), and n is the number of subjects positive (or negative) by the gold standard.[25] RPR and VDRL results were tabulated for samples that gave false negative rapid test results.

RESULTS

In Haiti, the Abbott Determine test was evaluated in 761 subjects (40 TPHA positive); the Omega Vistitect test in 516 subjects (55 TPHA positive); the Qualpro Syphicheck test in 543 subjects (41 TPHA positive); and the Standard Bioline test in 515 subjects (30 TPHA positive). A total of 2060 subjects were female, and 275 (12%) male; 704 of the women were pregnant (34%). Mean age was 28 years (range 18–64 years).

In China, the Abbott Determine test was evaluated in 445 subjects (83 TPHA positive); the Omega Visitect test in 445 subjects (83 TPHA positive); the Qualpro Syphicheck test in 415 subjects (89 TPHA positive); and the Standard Bioline test in 415 subjects (89 TPHA positive). Forty-four subjects (5%) were pregnant women, 71 (8%) attended for pre-marital testing, and the remainder attended with symptoms and/or signs of STIs. Six hundred and five subjects were male, and 255 female. Mean age was 34 years (range 18–61 years).

In Tanzania, the Abbott Determine test was evaluated in 528 subjects (57 TPHA positive); the Omega Visitect test in 528 subjects (57 TPHA positive); the Qualpro Syphicheck test in 582 subjects (55 TPHA positive); and the Standard Bioline test in 582 subjects (66 TPHA positive). All subjects were pregnant women attending for antenatal screening. Mean age was 24 years (range 13–45 years).

In Brazil, the Qualpro Syphicheck test was evaluated in 542 subjects (50 TPHA positive) and the Standard Bioline test in 542 subjects (50 TPHA positive). The Abbott Determine test was evaluated in 247 subjects (52 TPHA positive) and the Omega Vistitect test in 244 subjects (51 TPHA positive). In the first study (Syphicheck and Bioline), 72% of subjects were male and 28% female, and the mean age was 24 years. In the second study 44% were male and 56% female, and the mean age was 25 years. The higher prevalence of syphilis in the second study was caused by the inclusion of female sex workers who attended the clinic.

Tables 1–4 show the sensitivity and specificity of each rapid test at each study site, with 95% CI. Results are shown for whole blood tested in the clinic and in the laboratory, and for serum tested in the laboratory. The specificity of all four tests was >95% at each site, whether whole blood or serum was used, and whether the test was performed in the clinic or the laboratory. In nearly all cases the sensitivity was lower when whole blood was used rather than serum. The only exceptions to this were the Abbott Determine and Standard Bioline tests in Haiti, which were 100% sensitive using whole blood. These results should be interpreted with caution since the sample sizes were small (40 and 30 positives, respectively, by the gold standard test). Using serum, the sensitivity of the Standard Bioline exceeded 90% at all four sites, and the sensitivity of the Abbott Determine test exceeded 88% at all sites. The Qualpro Syphicheck was the least sensitive test, ranging from 67.4% sensitive in China to 97.6% in Haiti.

RPR results, with titres, were available from Tanzania and Haiti, and VDRL results from Brazil (table 5). The overall

Table 1 Sensitivity and specificity (±95% confidence intervals) of the Abbot Determine test

	Clinic: whole blood		Lab: whole blood		Lab: serum	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Tanzania	59.6 (12.7)	99.4 (0.7)	80.7 (10.2)	99.4 (0.7)	91.2 (7.3)	97.9 (1.3)
Brazil	88.5 (8.7)	97.9 (2.0)			88.5 (8.7)	97.9 (2.0)
China	81.9 (8.3)	99.4 (0.8)	77.1 (9.0)	100 (n = 362)	100 (n = 83)	98.9 (1.1)
Haiti	72.5 (13.8)	98.5 (0.9)	100 (n = 40)	95.7 (1.5)	100 (n = 40)	95.7 (1.5)

Table 2 Sensitivity and specificity (±95% confidence intervals) of the Omega Visitect test

	Clinic: whole blood		Lab: whole blood		Lab: serum	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Tanzania	75 (11.2)	99.8 (0.4)	80.7 (10.2)	99.6 (0.6)	84.2 (9.5)	99.1 (0.9)
Brazil	96.1 (5.3)	98.5 (1.7)			96.1 (5.3)	98.4 (1.7)
China	73.5 (9.5)	99.7 (0.6)	77.9 (8.9)	100 (n = 362)	94 (5.1)	98.1 (1.4)
Haiti	72.7 (11.8)	99.1 (0.9)	98.2 (3.5)	98.7 (1.0)	98.2 (3.5)	98.7 (1.0)

Table 3 Sensitivity and specificity (±95% confidence intervals) of the Qualpro Syphicheck test

	Clinic: whole blood		Lab: whole blood		Lab: serum	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Tanzania	78.6 (10.8)	99.1 (0.8)	85.4 (9.3)	99.1 (0.8)	87.3 (8.8)	98.9 (0.9)
Brazil	84.3 (10)	99.6 (0.6)			88.2 (8.9)	99.6 (0.6)
China	64 (10.0)	99.7 (0.6)	70.8 (9.4)	99.7 (0.6)	67.4 (9.7)	98.8 (1.2)
Haiti	80.5 (12.1)	97.8 (1.3)	97.6 (4.7)	98.6 (1.0)	97.6 (4.7)	98.4 (1.1)

Table 4 Sensitivity and specificity (±95% confidence intervals) of the Standard Bioline test

	Clinic: whole blood		Lab: whole blood		Lab: serum	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Tanzania	85.7 (8.4)	98.1 (1.2)	90.9 (6.9)	96.1 (1.6)	90.9 (6.9)	95.5 (1.8)
Brazil	88.2 (8.9)	99.4 (0.7)			90.2 (8.2)	99.4 (0.7)
China	87.6 (6.8)	99.4 (0.8)	87.6 (6.8)	99.4 (0.8)	95.5 (4.3)	97.9 (1.6)
Haiti	100 (n = 30)	98.3 (1.2)	100 (n = 30)	98.5 (1.1)	100 (n = 30)	98.5 (1.1)

prevalence of RPR or VDRL positivity among those with a positive gold standard treponemal test at the three sites were 53%, 69% and 79%, respectively. In Tanzania, 1 of 5 ''false negatives'' by the Abbott Determine test was RPR positive, at a titre of 1:16. Of 9, 7 and 6 false negatives by the other three tests, none was RPR positive. In Haiti, only 2 samples gave false negative results by rapid test (one each for the Omega Visitect and Qualpro syphicheck). Both were RPR negative. In Brazil 2 of 6 false negatives by Abbott Determine were VDRL positive (1:4 and 1:16); 1 of 2 false negatives by Omega Visitect (1:4); 3 of 6 false negatives by Qualpro Syphicheck (1:1, 1:1 and 1:32); and 3 of 5 false negatives by Standard Bioline (1:1, 1:1 and 1:32).

At all sites, agreement between the two readers in the clinic was >95%. In Brazil, there was perfect agreement between readers for the Abbott Determine and the Omega Visitect tests performed using whole blood in the clinic and serum in the laboratory. For the Qualpro Syphicheck and Standard Bioline tests there was 99% agreement. In Tanzania, agreements of 97.5%, 98.9%, 98.6% and 97.1% were obtained for the four tests, respectively, between whole blood results in the clinic and serum results in the laboratory.

DISCUSSION

The four tests we have evaluated compare favourably with standard treponemal tests for the diagnosis of syphilis. All are highly specific, and most show adequate sensitivity, at least when used with serum. The fact that they can be performed to a high standard on site in the clinic, by staff without laboratory training, and require no equipment or refrigeration means that

Table 5 Rapid plasma reagin (RPR) test results and titre for samples with positive gold standard and false negative rapid test

Country	Test	Sample	RPR	Titre
Tanzania	Abbott	1	Negative	
		2	Negative	
		3	Negative	
		4	Negative	
		5	Negative	1:16
Haiti	Omega	1	Negative	
	Qualpro	1	Negative	
Brazil	Abbott	1	Negative	
		2	Negative	
		3	Negative	
		4	Negative	
		5	Positive	1:4
		6	Positive	1:16
	Omega	1	Negative	
		2	Positive	1:4
	Qualpro	1	Negative	
		2	Negative	
		3	Negative	
		4	Positive	1:1
		5	Positive	1:1
		6	Positive	1:32
	Standard	1	Negative	
		2	Negative	
		3	Positive	1:1
		4	Positive	1:1
		5	Positive	1:32

they can be used in clinics without access to electricity. Their greatest value is likely to be in increasing the coverage of syphilis screening in rural areas of developing countries. On-site testing will also increase the proportion of cases treated when return rates are low. These tests could also complement syndromic management of STDs in resource-poor areas.

It is possible to separate serum in clinics with no electricity by waiting for blood to coagulate and for the clot to retract. The use of whole blood rather than serum would be preferable, since it would save time. Unfortunately the performance of these tests using whole blood was more variable than when using serum, with the sensitivity generally being lower when whole blood was used. The sensitivity with whole blood varied considerably between sites, suggesting that the low sensitivity was due to problems in reading and interpreting the test rather than to inherent properties of the tests themselves. They are clearly more difficult to read when whole blood is used.

In common with the gold standard treponemal tests used in this study, the rapid tests we evaluated do not distinguish between old, treated syphilis and syphilis requiring treatment. Clearly, the purpose of these tests is to identify those who need treatment. It is of no great concern if rapid tests give ''false negative'' results in subjects with old, treated syphilis. We therefore tested the hypothesis that subjects in whom the rapid test result was negative, but the gold standard positive, were likely to have negative RPR tests. The hypothesis was confirmed in Haiti, where both false negative results were RPR negative, and in Tanzania, where only one false negative sample was RPR positive. In Brazil, on the other hand, about half of the false negative samples were VDRL positive.

A rapid, point-of-care test which combined the properties of the reagin tests with those of treponemal tests would help to reduce the number of individuals treated unnecessarily, especially in populations with a high prevalence of treated syphilis.

Authors' affiliations

D Mabey, London School of Hygiene & Tropical Medicine, London, UK
R W Peeling, World Health Organization, Geneva, Switzerland
R Ballard, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
A S Benzaken, E Galbán, Fundação Alfredo da Matta, Manaus, Brasil
J Changalucha, D Everett, R Balira, National Institute for medical Research, Mwanza, Tanzania
D Fitzgerald, P Joseph, S Nerette, GHESKIO Centre, Port-au-Prince, Haiti
J Li, H Zheng, Peking Union Medical Collage, Beijing, China

Competing interests: none declared

REFERENCES

1 **Shakarishvili A**, Dubovskaya LK, Zohrabyan LS, *et al.* Sex work, drug use, HIV infection, and spread of sexually transmitted infections in Moscow, Russian Federation. *Lancet* 2005;**366**:57–60.
2 **Lin CC**, Gao X, Chen XS, *et al.* China's syphilis epidemic: a systematic review of seroprevalence studies. *Sex Transm Dis* 2006 May 31; [Epub ahead of print].
3 **Buchacz K**, Greenberg A, Onorato I, *et al.* Syphilis epidemics and human immunodeficiency virus (HIV) incidence among men who have sex with men in the United States: implications for HIV prevention. *Sex Transm Dis* 2005;**32**:S73–9.
4 **Gloyd S**, Chai S, Mercer MA. Antenatal syphilis in sub-Saharan Africa: missed opportunities for mortality reduction. *Health Policy Plan* 2001;**16**:29–34.
5 **Schmid G**. Economic and programmatic aspects of congenital syphilis prevention. *Bull World Health Organ* 2004;**82**:402–9.
6 **Fitzgerald DW**, Behets FM, Lucet C, *et al.* Prevalance, burden, and control of syphilis in Haiti's rural Artibonite region. *Int J Infect Dis* 1998;**2**:127–31.
7 **Southwick KL**, Blanco S, Santander A, *et al.* Maternal and congenital syphilis in Bolivia, 1996: prevalence and risk factors. *Bull World Health Organ* 2001;**79**:33–42.
8 **Watson-Jones D**, Changalucha J, Gumodoka B, *et al.* Syphilis and pregnancy outcomes in Tanzania. 1. Impact of maternal syphilis on outcome of pregnancy in Mwanza Region, Tanzania. *J Infect Dis* 2002;**186**:940–7.
9 **Connor N**, Roberts J, Nicoll A. Strategic options for antenatal screening for syphilis in the United Kingdom: a cost effectiveness analysis. *J Med Screen* 2000;**7**:7–13.
10 **Terris-Prestholt F**, Watson-Jones D, Mugeye K, *et al.* Is antenatal syphilis screening still cost-effective in Sub-Saharan Africa? *Sex Transm Inf* 2003;**79**:375–81.
11 **Temmerman M**, Mohamedali F, Fransen L. Syphilis prevention in pregnancy: an opportunity to improve reproductive and child health in Kenya. *Health Policy Plan* 1993;**8**:122–7.
12 **Fonn S**. A blood-result turn-around time survey to improve congenital syphilis prevention in rural area. *South African Medical Journal* 1996;**1**:67–71.
13 **Temmerman M**, Fonck K, Bashir F, *et al.* Declining syphilis prevalence in pregnant women in Nairobi since 1995: another success story in the STD field? *Int J STD AIDS* 1999;**10**:405–8.
14 **Hira SK**, Bhat GJ, Chikamata DM, *et al.* Syphilis intervention in pregnancy: Zambian demonstration project. *Genitourin Med* 1990;**66**:159–64.
15 **Rotchford K**, Lombard C, Zuma K, *et al.* Impact on perinatal mortality of missed opportunities to treat maternal syphilis in rural South Africa: baseline results from a clinic randomized controlled trial. *Tropical Medicine and International Health* 2000;**5**:800–4.
16 **Jenniskens F**, Obwaka E, Kirisuah S, *et al.* Syphilis control in pregnancy: decentralization of screening facilities to primary care level, a demonstration project in Nairobi, Kenya. *Int J Gynecol Obstet* 1995;**48**(suppl):S121–8.
17 **Fitzgerald DW**, Behets F, Preval J, *et al.* Decreased congenital syphilis incidence in Haiti's rural Artibonite Region following decentralized prenatal screening. *Am J Public Health* 2003;**93**:444–6.
18 **Majoko F**, Munjanja S, Nystrom L, *et al.* Field efficiency of syphilis screening in antenatal care: lessons from Gutu district of Zimbabwe. *Cent Afr J Med* 2003;**49**:90–3.
19 **West B**, Walraven G, Morison L, *et al.* Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa. *Sex Trans Dis* 2002;**78**:282–5.
20 **World Health Organization/Special Programme for Research and Training in Tropical Diseases (WHO/TDR)**. Laboratory-based evaluation of rapid syphilis diagnostics. Sexually Transmitted Diseases Diagnostics Initiative (SDI) Report: Diagnostics Evaluations Series No.1, 2003. www.who.int/std_diagnostics.
21 **Siedner M**, Zapitz V, Ishida M, *et al.* Performance of rapid syphilis tests in venous and fingerprick whole blood specimens. *Sex Transm Dis* 2004;**31**:9.
22 **Diaz T**, Almeida MG, Georg I, *et al.* Evaluation of the Determine Rapid Syphilis TP assay using sera. *Clin Diagn Lab Immunol* 2004;**11**:98–101.
23 **Fears MB**, Pope V. Syphilis fast latex agglutination test, a rapid confirmatory test. *Clin Diagn Lab Immunol* 2001;**8**:841–2.
24 **Herring AJ**, Ballard RC, Pope V, *et al.* A multi-centre evaluation of nine rapid, point-of-care syphilis tests using archived sera. *Sex Transm Infect* 2006;**82**(suppl v):v7–12.
25 **Smith PG**, Morrow RH. *Field trials of health interventions in developing countries: a toolbox*, 2nd ed.Macmillan, London, 1996.