ARTICLE

Comparison of Enzyme-Linked Immunospot Assay and Tuberculin Skin Test in Healthy Children Exposed to *Mycobacterium tuberculosis*

Philip C. Hill, MPH, FRACP[a], Roger H. Brookes, PhD[a], Ifedayo M.O. Adetifa, MB, BS, FWACP[a], Annette Fox, PhD[a], Dolly Jackson-Sillah, MB, BS, MSc[a], Moses D. Lugos, FIMLS[a], Simon A. Donkor, BSc[a], Roger J. Marshall, PhD[b], Stephen R.C. Howie, MBCHB, FRACP[c], Tumani Corrah, PhD, FRCP[a], David J. Jeffries, PhD[a], Richard A. Adegbola, PhD, FRCPath[a], Keith P.W.J. McAdam, FRCP[d]

[a]Tuberculosis Division, [c]Bacterial Diseases Programme, and [d]Infectious Diseases Institute, Mulago Hospital Complex, Kampala, Uganda; [b]School of Population Health, University of Auckland, Auckland, New Zealand

Financial Disclosure: Dr Brookes has a patent relating to ex vivo enzyme-linked immunospot that is licensed through Oxford University.

ABSTRACT

OBJECTIVE. To compare the enzyme-linked immunospot (ELISPOT) assay with the tuberculin skin test (TST) in children for the diagnosis of *Mycobacterium tuberculosis* infection in the Gambia.

METHODS. We divided child contacts of sputum smear-positive tuberculosis cases into 3 age categories (<5, 5–9, and 10–14 years) and assessed agreement between the 2 tests plus their relationship to prior Bacille Calmette-Guerin (BCG) vaccination. We categorized a child's level of *M tuberculosis* exposure according to where he/she slept relative to a case: the same room, same house, or a different house. The relationship between exposure and test result was assessed by multiple logistic regression.

RESULTS. In child contacts of 287 cases, 225 (32.5%) of 693 were positive by TST and 232 (32.3%) of 718 by ELISPOT. The overall agreement between tests was 83% and the discordance was not significant. Both tests responded to the *M tuberculosis* exposure gradient in each age category. The percentage of those who were TST positive/ELISPOT negative increased with increasing exposure. At the lowest exposure level, the percentage of ELISPOT-positive children who were TST negative was increased compared with the highest exposure level. Neither test had evidence of false positive results because of BCG.

CONCLUSIONS. In Gambian children, the ELISPOT is slightly less sensitive than the TST in the diagnosis of *M tuberculosis* infection from recent exposure, and neither test is confounded by prior BCG vaccination. Evidence of reduced TST sensitivity in subjects with the lowest known recent *M tuberculosis* exposure suggests that, when maximal sensitivity is important, the 2 tests may be best used together.

www.pediatrics.org/cgi/doi/10.1542/peds.2005-2095

doi:10.1542/peds.2005-2095

Key Words
tuberculin skin test, ELISPOT, tuberculosis, Mycobacterium tuberculosis

Abbreviations
TST—tuberculin skin test
BCG—Bacille Calmette-Guerin
ESAT-6 — early secretory antigenic target 6
CFP-10 — culture filterate protein 10
ELISPOT— enzyme-linked immunospot
MRC—Medical Research Council
SFU—spot forming units
OR— odds ratio
CI— confidence interval

Accepted for publication Nov 3, 2005

Address correspondence to Philip Hill, MPH, FRACP, Medical Research Council Laboratories, PO Box 273, Banjul, Gambia. E-mail: phill@mrc.gm

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

TUBERCULOSIS IN CHILDREN is an important, but neglected, global health problem.[1] It was estimated in 1991 that, from a reservoir of 180 million children with primary or latent *Mycobacterium tuberculosis* infection, there were 1.3 million cases of childhood tuberculosis and 450 000 deaths.[2] The overwhelming majority of these cases occur in developing countries. Children with tuberculosis tend not to be the focus of public health strategies against the disease, because >95% have negative sputum smears and do not contribute substantially to the immediate course of the epidemic.[3] However, many adults who develop smear-positive disease acquire a primary infection during childhood.[4]

Tracing is recommended in childhood contacts of adult tuberculosis cases so that they can receive treatment for latent tuberculosis infection.[5] However, such treatment is rare in developing countries because of lack of resources, poor diagnostic tools, and fear of giving monotherapy to children who actually have disease. The tuberculin skin test (TST) is the only universally used method for detecting *M tuberculosis* infection, for which it has unknown sensitivity and specificity, the latter being compromised by cross-reactivity with antigens of environmental mycobacteria and bacille Calmette-Guerin (BCG) vaccine. It is also subject to the booster phenomenon, reader variability, and false-negative results in immune compromise.[6–8] Recently, T-cell interferon γ responses to 2 relatively specific *M tuberculosis* antigens, early secretory antigenic target 6 (ESAT-6) and culture filterate protein 10 (CFP-10), have been investigated for diagnosis of *M tuberculosis* infection.[9–11] In children, this enzyme-linked immunospot (ELISPOT) assay has been reported to correlate more closely with *M tuberculosis* exposure and to be unaffected by BCG vaccination.[12] We reported recently that an ELISPOT assay offered increased specificity in the diagnosis of *M tuberculosis* infection in the Gambia, at the cost of some sensitivity.[13] To more fully evaluate this assay against the TST in children, we expanded our study and present a detailed comparison in tuberculosis case contacts under the age of 15 years in a tuberculosis-endemic tropical setting.

METHODS

Participants

Sputum smear-positive tuberculosis index cases >15 years of age were recruited in Greater Banjul as described previously.[13] Included cases had 2 sputum samples positive for acid-fast bacilli by Ziehl-Neelson stain and *M tuberculosis* on culture. They were identified at the major government health centers and the (Medical Research Council [MRC] laboratories) outpatients' clinic, where, after counseling, they had an HIV test.

Household contacts were included if they were between 6 months and 14 years of age and lived for ≥3 months in the same compound as a case. They were not eligible if treated for tuberculosis in the past year and were excluded if diagnosed with tuberculosis within 1 month of recruitment. Subjects were brought to the MRC laboratories, invited to give informed consent, interviewed, and examined, and a blood sample was taken for ELISPOT and HIV test. Fresh samples from all of the participants were processed onsite. On any particular day, some subjects were randomly excluded from having an ELISPOT test if laboratory capacity had been reached.

Contacts underwent a purified protein derivative skin test (2 tuberculin units, purified protein derivative RT23, Statens Serum Institut, Copenhagen, Denmark), administered by a trained field worker. Induration was measured at 48–72 hours. A second independent reading was conducted, for quality control, in 107 contacts, giving a κ statistic of 0.86 and 93% concordance. Subjects with a positive skin test (mean induration diameter: ≥10 mm) were offered a chest radiograph, and those with symptoms underwent an assessment using a standardized form and clinical examination.

Those with tuberculosis disease were referred to the National Tuberculosis Control Program for free treatment. There is no current practice of preventive treatment in the Gambia. The Gambia Government/MRC Joint Ethics Committee approved the study.

Laboratory Procedures

Sputum smears were prepared and stained with auramine-phenol[14] and confirmed by Ziehl-Neelsen test. Decontaminated specimens were inoculated into 1 slope each of Lowenstein-Jensen medium containing glycerol and sodium pyruvate, respectively, and 1 vial of BACTEC 9000 MB medium for isolation of *M tuberculosis*. All of the mycobacterial cultures were identified and confirmed by using standard procedures.

The ex vivo ELISPOT assays for interferon-γ were performed as described previously.[15] For this study, synthetic, sequential peptides spanning the length of ESAT-6 and CFP-10 (ABC, Imperial College, London, United Kingdom) were used. Each peptide was 15 amino acids long and overlapped its adjacent peptide by 10 residues. ESAT-6 and CFP-10 peptide pools were used at 5 μg/mL. The positive control was phytohemaglutinin (Sigma-Aldrich, Dorset, United Kingdom). All of the antigens were tested in duplicate wells.

Assays were counted with an automated ELISPOT reader (AID-GmbH, Strassberg, Germany). The spot forming unit (SFU) numbers counted in each well were automatically entered into a database. Supplementary details were added by double data entry. Positive test wells were predefined as containing ≥8 SFUs (40 SFU/million cells) more than negative control wells.[16] For a positive ESAT-6/CFP-10 result, it was necessary for 1 or more pools of overlapping peptides to be positive. Phytohemaglutinin wells were set to ≥150 SFUs above neg-

ative control wells. Negative control wells were required to have <20 SFUs.

Testing for HIV-1 or HIV-2 infection was by competitive enzyme-linked immunosorbent assays (Wellcome Laboratories, Kent, United Kingdom) and Western blot (Diagnostics Pasteur, Marnes-la-Coquette, France).

Ascertainment of Exposure

Tuberculosis contacts were categorized according to where they slept: in the same bedroom as the case, a different bedroom in the same house, or in a different house in the same compound. As described previously,[17,18] we also assessed self-reported duration of cough and sputum smear grade.

Data Management and Analysis

All of the data were entered using double data entry into a Microsoft Access database (Microsoft, Redmond, WA) and checked for errors. The concordance between the ELISPOT and TST was assessed by the calculation of a κ statistic and the discordance by McNemar's test. A random-effects logistic regression model, taking into account household clustering, was used to assess the relationship between sleeping proximity to an index case and test results. Age and gender were included in the analysis at the outset. Other variables assessed for possible inclusion in the model were ethnicity, BCG scar status, and duration of cough of the respective index case. The likelihood ratio test was used to test for linear trend and for interaction between variables. All of the statistical analyses were conducted using Stata 8 software (Stata Corp, College Station, TX).

RESULTS

From June 19, 2002, until September 2004, 1132 children were recruited from the households of 287 tuberculosis cases; 917 were selected and eligible for the study. Of these, 856 had an adequate sample taken, from whom 718 (83%) had an ELISPOT result that met the criteria for analysis. Just over half of the children were male, and only 3 of 711 tested were HIV positive. The HIV-positive children were not excluded from the analysis (Table 1).

Overall, 232 (32.3%) of 718 children had a positive ELISPOT result, and 225 (32.5%) of 693 with a TST that was read, had a positive result. Figure 1 shows the numbers of contacts positive for the 2 tests in relation to each other ($n = 693$) using a scaled rectangle diagram,[19] where the numbers of individuals are represented by rectangles of a size that correlate with their relative proportion. Sixty (26.7%) of the TST-positive children were ELISPOT negative, and 55 (25%) who had a positive ELISPOT result were TST negative. The overall agreement between the 2 tests was 83% ($\kappa = 0.62$), and the discordance was not statistically significant ($P = .64$).

The univariate odds of a positive test result across the *M tuberculosis* exposure categories (represented as sleeping proximity to a case) for each of 3 age groups are shown in Table 2. Both tests were significantly likely to be more positive with increasing exposure to the index

TABLE 1 Characteristics of 718 Childhood Contacts of Tuberculosis Patients in the Gambia

Characteristics	Sleeping Proximity Gradient			All ($n = 718$)
	Separate House ($n = 182$)	Separate Room ($n = 372$)	Same Room ($n = 163$)	
Age				
Mean	8.2	7.7	6.4	7.5
Median (range)	8 (0.9–14)	7.5 (0.5–14)	6 (0.5–14)	7.0 (0.5–14)
0.5–4 y, *n* (%)	39 (21.3)	90 (24.2)	66 (40.5)	195 (27.2)
5–9 y, *n* (%)	66 (36.1)	148 (39.8)	61 (37.4)	275 (38.3)
10–14 y, *n* (%)	78 (42.6)	134 (36.0)	36 (22.1)	248 (34.5)
Gender, *n* (%)				
Male	94 (51.4)	190 (51.1)	95 (58.3)	379 (52.8)
Female	89 (48.6)	182 (48.9)	68 (41.7)	339 (47.2)
Ethnic group, *n* (%)				
Madinka	64 (35.0)	127 (34.1)	55 (33.7)	246 (34.3)
Jola	41 (22.4)	88 (23.7)	33 (20.3)	162 (22.6)
Wollof	29 (15.9)	52 (14.0)	21 (12.9)	102 (14.2)
Fula	13 (7.0)	30 (8.1)	14 (8.6)	57 (7.9)
Others	36 (19.7)	75 (20.1)	40 (24.5)	151 (21.0)
Clinical findings				
BCG scar, *n* (%)				
Absent	81 (44.3)	166 (44.6)	66 (40.5)	313 (43.6)
Present	85 (46.5)	165 (44.4)	80 (49.1)	330 (46.0)
Uncertain	17 (9.2)	41 (11.0)	17 (10.4)	75 (10.4)
HIV results, *n* (%)				
Positive	2 (1.1)	1 (0.3)	0 (0)	3 (100.0)

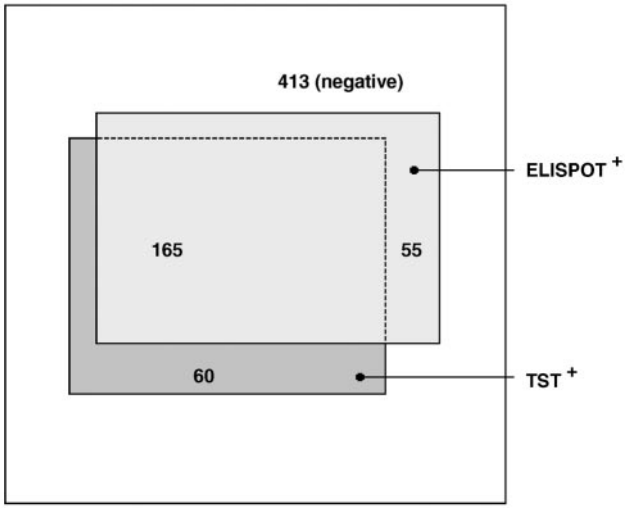

FIGURE 1
Scaled rectangle diagram of the number of children (rectangle size correlates with relative proportion) with each combination of ELISPOT and TST results ($n = 693$).

case in all of the age groups. This was most marked in the <5-years age group and for the TST. In the multivariable analyses, adjusting for age, gender, and ethnicity, the changes in positivity across the exposure gradient remained significant for both the ELISPOT and TST.

A BCG scar was clearly visible in 330 (46.0%) of the children (Table 1). The univariate odds of a positive test for those who were BCG scar positive, compared with those with no evidence of a scar, was odds ratio (OR) at 0.8 (95% confidence interval [CI]: 0.5–1.2; $P = .34$) for ELISPOT and OR at 0.8 (95% CI: 0.5–1.2; $P = .27$) for TST. When adjusted for age, gender, and ethnicity in a multivariable analysis, the effect of the presence of a BCG scar on the results for both tests remained nonsignificant. A separate analysis of <5-year-old children (most recently exposed to BCG vaccine) also showed no significant relationship between scar status and test results (OR: 2.5; 95% CI: 0.6–11.0; $P = .21$ for ELISPOT and OR: 1.7; 95% CI: 0.4–6.7; $P = .45$ for TST).

The odds of test positivity were not increased in those contacts of a tuberculosis case with sputum smear grade 3+ compared with contacts of a case with sputum smear grade 1+ (OR: 1.1; 95% CI: 0.3–4.9 for ELISPOT and OR: 1.7; 95% CI: 0.3–8.5 for TST). Duration of cough of >10 weeks in a respective index case also did not increase the odds of a positive test compared with cough duration of 0–4 weeks (OR: 1.1; 95% CI: 0.6–2.2 for ELISPOT and OR: 1.5; 95% CI: 0.7–3.2 for TST).

The exposure gradient by sleeping proximity enabled a stratified analysis of the level of concordance between the 2 tests. In all of the age groups, there was a "divergence" between TST and ELISPOT results with increasing exposure to the index case (Fig 2). Consistent with this divergence, the number of contacts who had positive TST and negative ELISPOT results increased from 3.3% of those sleeping in a different house to 12.3% of those sleeping in the same room ($P = .006$). This compared with 7.7% and 6.1%, respectively, across the exposure gradient for the subgroup that was ELISPOT-positive and TST negative ($P = .7$). Analysis of TST results in the subgroup of children who were ELISPOT positive showed that there was an important change in the number of ELISPOT-positive children who were TST negative across the exposure gradient: whereas 13.7% of ELISPOT-positive children in the highest exposure gradient were TST negative, 40% of ELISPOT-positive children sleeping in a different house as a case were TST negative ($P = .008$).

DISCUSSION

As far as we are aware, this study provides the first large-scale comparison of the ELISPOT to the TST for the diagnosis of *M tuberculosis* infection in childhood contacts of tuberculosis cases in a tuberculosis-endemic tropical setting. The tests were relatively concordant with each other, with no statistically significant discordance overall. In all of the 3 age categories assessed, both tests responded to a gradient of recent exposure to *M tuberculosis* infection, the TST most dramatically. Neither test showed evidence of false-positive results in the presence of a BCG scar, even in <5-year-old children. An increase in the proportion of children who were TST positive/ELISPOT negative with increasing exposure suggests lower sensitivity of the ELISPOT for the detection of *M tuberculosis* infection from recent exposure compared with the TST. In contrast, a large proportion (40%) of ELISPOT-positive children in the lowest exposure group were TST negative, implying a possible sensitivity problem for the TST in those most similar to the general community.

Our study demonstrates the usefulness of an exposure gradient when assessing the performance of diagnostic tests for *M tuberculosis* infection in the absence of a gold standard. Although only 8% of contacts were ELISPOT positive and TST negative overall, the finding that the proportion of children with this result increased with increasing *M tuberculosis* exposure (3.3–12.3%) is consistent with our previous finding in all of the age groups[13] and the assertion that 2 antigens may not offer adequate sensitivity.[20] The ELISPOT test is not as sensitive as the TST for the diagnosis of *M tuberculosis* infection from known recent *M tuberculosis* exposure.

Conversely, for those in the lowest exposure group, we found evidence of a sensitivity problem for the TST. The reasons for the increasing discordance between the TST and ELISPOT with decreasing known *M tuberculosis* exposure are likely to be complex, requiring a detailed understanding of the mechanisms behind each type of immune response and repeated comparisons over time (ie, longitudinal as well as cross-sectional data). Only

TABLE 2 **Univariable and Multivariable ORs Determined by Logistic Regression (Household as Random Effect) for Sleeping Proximity as a Surrogate Marker of Exposure to *M tuberculosis* by Age Group**

Sleep Proximity by Age group	ESAT-6/CFP-10 ELISPOT ($n = 718$)			TST ($n = 693$)		
	Positive Results, *n* (%) of Contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Positive Results, *n* (%) of Contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
0.5–4 y						
Different house	4 (10.3)	1	1	4 (10.0)	1	1
Different room	27 (30.0)	5.9 (1.2–28.3)	5.4 (1.2–24.4)	27 (31.4)	7.4 (1.3–42.9)	8.7 (1.3–58.6)
Same room	30 (45.5)	12.2 (2.4–62.0)	11.6 (2.3–57.9)[a]	35 (55.6)	26.7 (3.9–184.6)	38.3 (4.3–341.8)[b]
5–9 y						
Different house	14 (21.2)	1	1	11 (16.9)	1	1
Different room	48 (32.4)	2.0 (0.8–4.6)	1.9 (0.8–4.4)	43 (29.7)	3.3 (0.9–12.6)	3.2 (0.8–12.4)
Same room	27 (44.3)	3.5 (1.3–9.5)	3.5 (1.3–9.4)[c]	31 (52.5)	16.1 (3.1–82.2)	15.9 (3.1–81.2)[b]
10–14 y						
Different house	18 (23.1)	1	1	12 (16.2)	1	1
Different room	46 (34.3)	2.3 (0.8–6.3)	2.3 (0.8–6.4)	45 (60.8)	3.8 (1.5–10.0)	3.8 (1.5–9.6)
Same room	18 (50.0)	5.9 (1.4–24.3)	6.5 (1.5–27.7)[c]	17 (23.0)	7.4 (2.0–26.8)	8.0 (2.3–28.5)[b]
Overall						
Different house	36 (19.7)	1	1	27 (15.0)	1	1
Different room	121 (32.5)	2.1 (1.1–3.8)	2.1 (1.1–3.8)	115 (32.4)	3.6 (1.8–7.0)	3.4 (1.8–6.6)
Same room	75 (46.0)	3.9 (2.0–7.7)	4.6 (2.3–9.3)[a]	83 (52.5)	9.2 (4.3–19.6)	10.2 (4.7–21.8)[b]

See text for description of explanatory variables used. TST positive result defined as a mean induration diameter of ≥10 mm.

[a] $P < .01$ for linear trend.

[b] $P < .001$ for linear trend.

[c] $P < .05$ for linear trend.

~32% of all contacts were positive for either TST or ELISPOT in our setting with endemic tuberculosis and intense exposure to environmental mycobacteria, suggesting that the immune response to both of these tests wanes over time. The chance that the kinetics of waning for the 2 tests are identical is very small. The ex vivo ELISPOT detects recently activated lymphocytes with immediate effector function and effector memory cells that are present transiently.[21] Whereas ESAT-6 and CFP-10 are secreted mainly early in infection,[22] it is likely that *M tuberculosis* predominantly secretes other antigens at different times. There is a need to identify the full repertoire of secreted antigens during the life cycle of the organism. Other antigens, such as the Esx-1 secretion-associated protein A,[23] may, in combination or alone, provide improved sensitivity and specificity in the diagnosis of *M tuberculosis* infection. However, in dormancy, there may be periods of time where no antigens at all are secreted. It is likely that ELISPOT and TST conversion and reversion occur at different rates over time, explaining some of our findings.[24] A longitudinal comparison of the 2 tests is underway in the Gambia.

The absence of any significant effect of BCG vaccine on the TST is consistent with our previous results and those from other tropical settings[25–27] but inconsistent with a recent meta-analysis, which reported increased likelihood of a positive TST in BCG-vaccinated persons, in particular, within 15 years after vaccination.[28] The absence of a significant effect of BCG scar status on the ELISPOT with age was not unexpected. A weakness of our study and most others (including the majority of those in the above-mentioned meta-analysis) is that they rely on the presence or absence of a BCG scar because of the difficulties in obtaining accurate immunization histories and/or records. Considering that 6–17% of BCG-vaccinated children may not develop scars after vaccination, the calculation of any effect of BCG on test results may be an underestimate.[29,30] Furthermore, it is possible that BCG protects against the development of new infection after exposure, which might also lead to an underestimate of the presence of false-positive results. Therefore, a small benefit of ELISPOT over the TST with respect to the confounding effect of BCG vaccination in our setting cannot be excluded. An even larger study would be required to resolve this issue.

A blood test for *M tuberculosis* has some theoretical advantages, even in developing countries. For example, it is a test with a result within 24 hours that does not require 2 patient visits. Furthermore, it does not involve the injection of antigens that may boost the results of future tests. However, the capital outlay needed to set up an appropriately equipped laboratory and employ adequately skilled staff is a significant impediment to the routine use of the ELISPOT test in developing countries. It is also subject to technical failure in a small proportion of individuals. The use of whole blood assays may be a partial solution to these problems. However, this study has shown that, irrespective of these issues, a T-cell assay using *M tuberculosis*-specific antigens is not as sensitive for the diagnosis of *M tuberculosis* from recent exposure as the TST in the Gambia and offers no added benefit

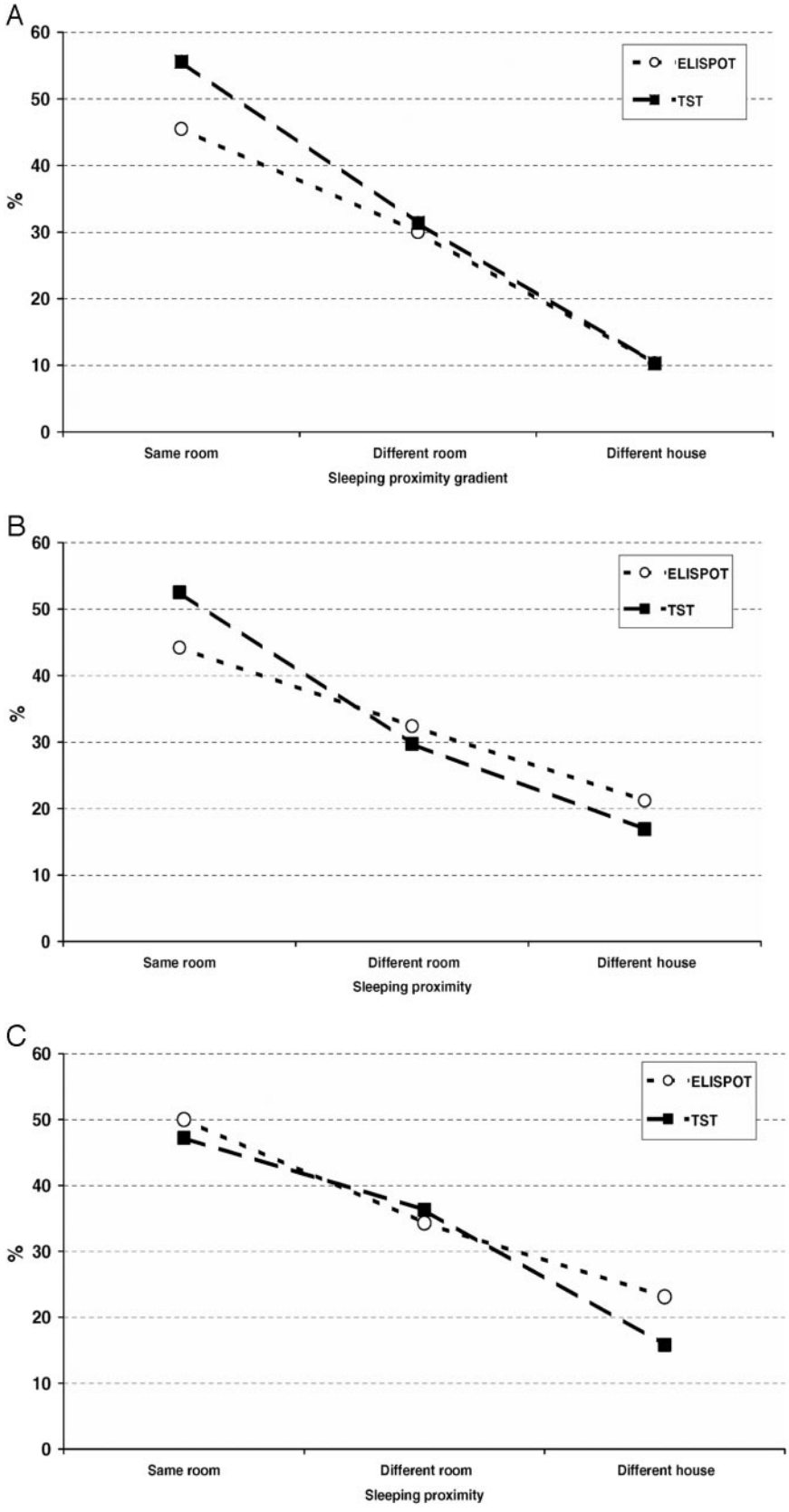

FIGURE 2
Percentage of children positive for ELISPOT and TST by *M tuberculosis* exposure (according to sleeping proximity to a tuberculosis case), by age group. A, Age group 0–4 years ($n = 195$); B, Age group 5–9 years ($n = 275$); C, Age group 10–14 years ($n = 248$).

with respect to the confounding effect of BCG vaccination. However, for those (eg, vaccine trialists) wishing to identify individuals in the general community with any evidence of *M tuberculosis* infection in similar settings, a T-cell assay as part of the screening procedures, in addition to a TST, is advisable at the present time.

ACKNOWLEDGMENTS

This study was funded by the Medical Research Council (United Kingdom) and the European Commission (Africa Tuberculosis Vaccine Consortia, grant QLRT-01613; Longitudinal Study of Correlates of Tuberculosis Protection in human contacts, grant ICFP599A4PR01).

We thank the National Tuberculosis Control Program for their ongoing collaboration and the Medical Research Council field staff and laboratory assistants for their hard work.

REFERENCES

1. Walls T, Shingadia D. Global epidemiology of paediatric tuberculosis. *J Infect.* 2004;48:13–22
2. Dolin P, Raviglione M, Kochi A. Global tuberculosis incidence and mortality during 1990–2000. *Bull World Health Organ.* 1994;72:213–220
3. Starke JR. Tuberculosis in children. In: Reichman LB, Hershfield ES, eds. *Tuberculosis: A Comprehensive International Approach*. New York, NY: Marcel Dekker Inc; 1993:329–367
4. Starke JR. Paediatric tuberculosis: time for a new approach. *Tuberculosis.* 2003;83:208–212
5. World Health Organization. *Treatment of Tuberculosis: Guidelines for National Programmes*. Geneva, Switzerland: World Health Organization; 2003
6. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. *Clin Infect Dis.* 1993;17:968–975
7. Black GF, Weir RE, Floyd S, et al. BCG-induced increase in interferon gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. *Lancet.* 2002;359:304–307
8. Jasmer RM, Nahid P, Hopewell PC. Clinical practice: latent tuberculosis infection. *N Engl J Med.* 2002;359:1393–1401
9. Demisse A, Ravn P, Olobo J, et al. T-cell recognition of *Mycobacterium tuberculosis* culture filtrate fractions in tuberculosis patients, and their household contacts. *Infect Immunol.* 1999;67:5967–5971
10. Vekemans J, Lienhardt C, Sillah JS, et al. Tuberculosis contacts but not patients have higher gamma interferon responses to ESAT-6 than do community controls in the Gambia. *Infect Immunol.* 2001;69:6554–6557
11. Lalvani A, Nagvenkar P, Udwadia Z, et al. Enumeration of T cells specific for RD 1 encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. *J Infect Dis.* 2001;183:469–477
12. Ewer K, Deeks J, Alvarez L, et al. Comparison of T-cell based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet.* 2003;361:1168–1173
13. Hill PC, Brookes RH, Fox A, et al. Large-scale evaluation of enzyme-linked immunospot assay and skin test for the diagnosis of *Mycobacterium tuberculosis* infection against a gradient of exposure in the Gambia. *Clin Infect Dis.* 2004;38:966–973
14. Heifets LB, Good RB. Current laboratory methods for the diagnosis of tuberculosis. In: Bloom BR, ed. *Tuberculosis: Protection, Pathogenesis, and Control*. Washington, DC: American Society for Microbiology; 1994:85–110
15. Lalvani A, Brookes R, Hambleton S, Britton WJ, Hill AV, McMichael AJ. Rapid effector function in CD+ memory T cells. *J Exp Med.* 1997;186:859–865
16. Jeffries DJ, Hill PC, Fox A, et al. Determination of ELISPOT assay and PPD skin test cut-offs for the diagnosis of *Mycobacterium tuberculosis* in the Gambia. *Int J Tuberc Lung Dis.* 2006;10:192–198
17. Lienhardt C, Fielding K, Sillah J, et al. Risk factors for tuberculosis infection in sub-saharan Africa: a contact study in The Gambia. *Am J Respir Crit Care Med.* 2003;168:448–455
18. Lienhardt C, Sillah J, Kielding K, et al. Risk factors for tuberculosis in children in contact with infectious tuberculosis cases in the Gambia, West Africa. *Pediatrics*. 2003;111(5). Available at: www.pediatrics.org/cgi/content/full/111/5/e608
19. Marshall RJ. Displaying clinical data relationships using scaled rectangle diagrams. *Stat Med.* 2001;20:1077–1088
20. Ulrichs T, Munk ME, Mollenkopf H, et al. Differential T cell responses to *Mycobaterium tuberculosis* ESAT6 in tuberculosis patients and healthy donors. *Eur J Immunol.* 1998;28:3949–3958
21. Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen specific IFN-γ-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: associations with clinical disease state and effect of treatment. *J Immunol.* 2001;167:5217–5225
22. Haile Y, Bjune G, Wiker HG. Expression of the mceA, esat-6 and hspX genes in *Mycobacterium tuberculosis* and their responses to aerobic conditions and to restricted oxygen supply. *Microbiology.* 2002;148:3881–3886
23. Fortune SM, Jaeger A, Sarracino DA, et al. Mutually dependent secretion of proteins required for mycobacterial virulence. *PNAS.* 2005;102:10676–10681
24. Fine PEM, Bruce J, Ponninghaus JM, Nkhosa P, Harawa A, Vynnycky E. Tuberculin sensitivity: conversions and reversions in a rural African population. *Int J Tuberc Lung Dis.* 1999;3:962–975
25. Mudido PM, Guwatudde D, Nakakeeto MK, et al. The effect of Bacille Calmette-Guerin vaccination at birth on tuberculin skin reactivity in Ugandan children. *Int J Tuberc Lung Dis.* 1999;3:891–895
26. Almeida LM, Barbieri MA, Da Paixao AC, et al. Use of the purified protein derivative to assess the risk of children in close contact with adults with tuberculosis in a population with high Calmette-Guerin bacillus coverage. *Pediatr Infect Dis J.* 2001;20:1061–1065
27. Pai M, Kaustubh G, Rajnish J, et al. *Mycobacterium tuberculosis* infection in health care workers in rural India. Comparison of a whole blood interferon gamma assay with tuberculin skin testing. *JAMA.* 2005;293:2746–2755
28. Wang L, Turner MO, Elwod RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Calmette Guerin vaccination in tuberculin skin test measurements. *Thorax.* 2002;57:804–809
29. Guwatudde D, Nakakeeto M, Jones-Lopez EC, et al. Tuberculosis in household contacts of infectious cases in Kampala, Uganda. *Am J Epidemiol.* 2003;158:887–898
30. Young TK, Mirdad S. Determinants of tuberculin sensitivity in a child population covered by mass BCG vaccination. *Tuber Lung Dis.* 1992;73:94–100

Comparison of Enzyme-Linked Immunospot Assay and Tuberculin Skin Test in Healthy Children Exposed to *Mycobacterium tuberculosis*

Philip C. Hill, Roger H. Brookes, Ifedayo M.O. Adetifa, Annette Fox, Dolly Jackson-Sillah, Moses D. Lugos, Simon A. Donkor, Roger J. Marshall, Stephen R.C. Howie, Tumani Corrah, David J. Jeffries, Richard A. Adegbola and Keith P.W.J. McAdam

Pediatrics 2006;117;1542
DOI: 10.1542/peds.2005-2095

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Comparison of Enzyme-Linked Immunospot Assay and Tuberculin Skin Test in Healthy Children Exposed to *Mycobacterium tuberculosis*

Philip C. Hill, Roger H. Brookes, Ifedayo M.O. Adetifa, Annette Fox, Dolly Jackson-Sillah, Moses D. Lugos, Simon A. Donkor, Roger J. Marshall, Stephen R.C. Howie, Tumani Corrah, David J. Jeffries, Richard A. Adegbola and Keith P.W.J. McAdam

Pediatrics 2006;117;1542
DOI: 10.1542/peds.2005-2095