

**Symposium 30. The challenge of TB
laboratory diagnosis in the HIV-infected**
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Diagnosing latent tuberculosis in the HIV-infected in high TB prevalence settings

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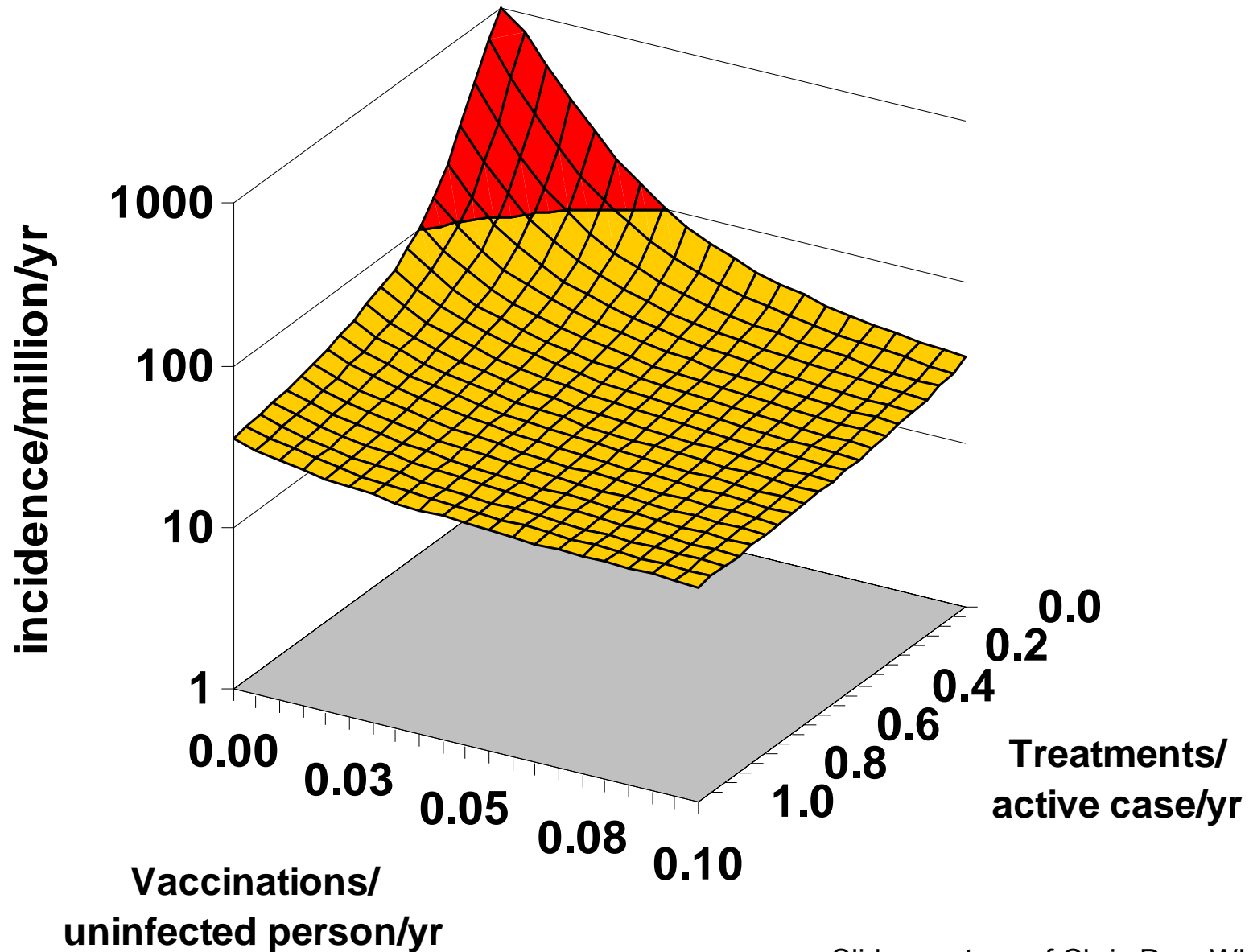
Introduction

Why diagnose LTBI in high-prevalence settings?

- Current approach to TB control in high-endemicity settings is on improving active case detection & on the provision and adherence to treatment¹
- However, the diagnosis of LTBI is recommended in high-endemicity settings in the HIV-infected², because:
 - It is a group of particularly high-risk of reactivation to active TB. The lifetime risk of developing active TB following infection is estimated as 50%². Yearly risk estimates varying from 6-16%³.
 - Preventative treatment known to be effective in reducing subsequent development of active TB^{4,5}
 - More generally, awareness of TB status (active or latent) becomes increasingly important with more widespread use of ARTs due to the risk of Immune Reconstitution Syndrome⁶
- More generally, the diagnosis of LTBI in high-endemicity settings is likely to grow over time; as new modelling has shown that current approaches alone will not eradicate TB⁷
 - More widespread screening and treatment for LTBI will be crucial for the long-term eradication of TB in high-endemicity settings.
- The ability to effectively diagnose LTBI in HIV-infected people is thus of major current importance to reduce TB-associated mortality and for the eventual eradication of TB

ELIMINATING TB BY 2050

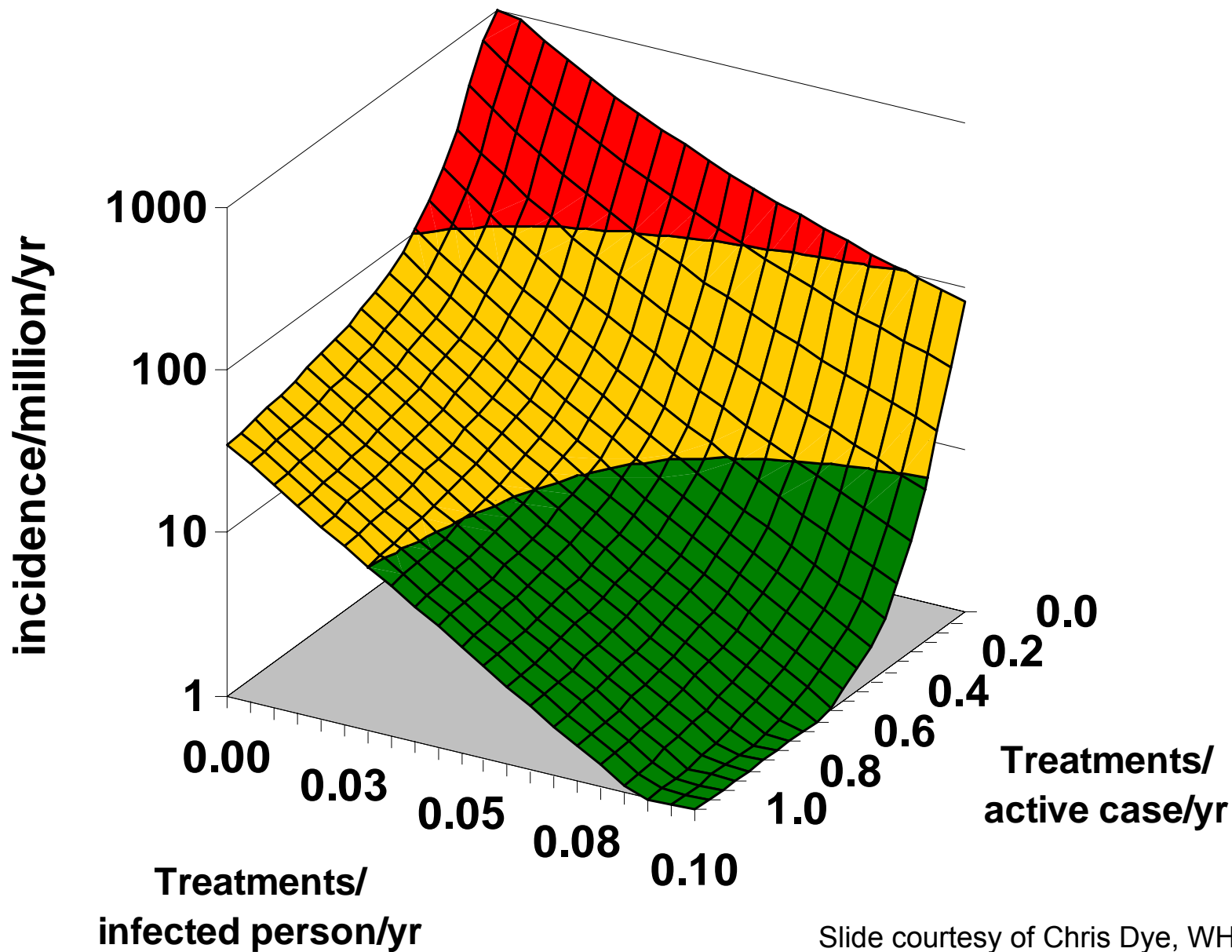
NOT BY TREATING ACTIVE DISEASE AND VACCINATION



Slide courtesy of Chris Dye, WHO, Geneva

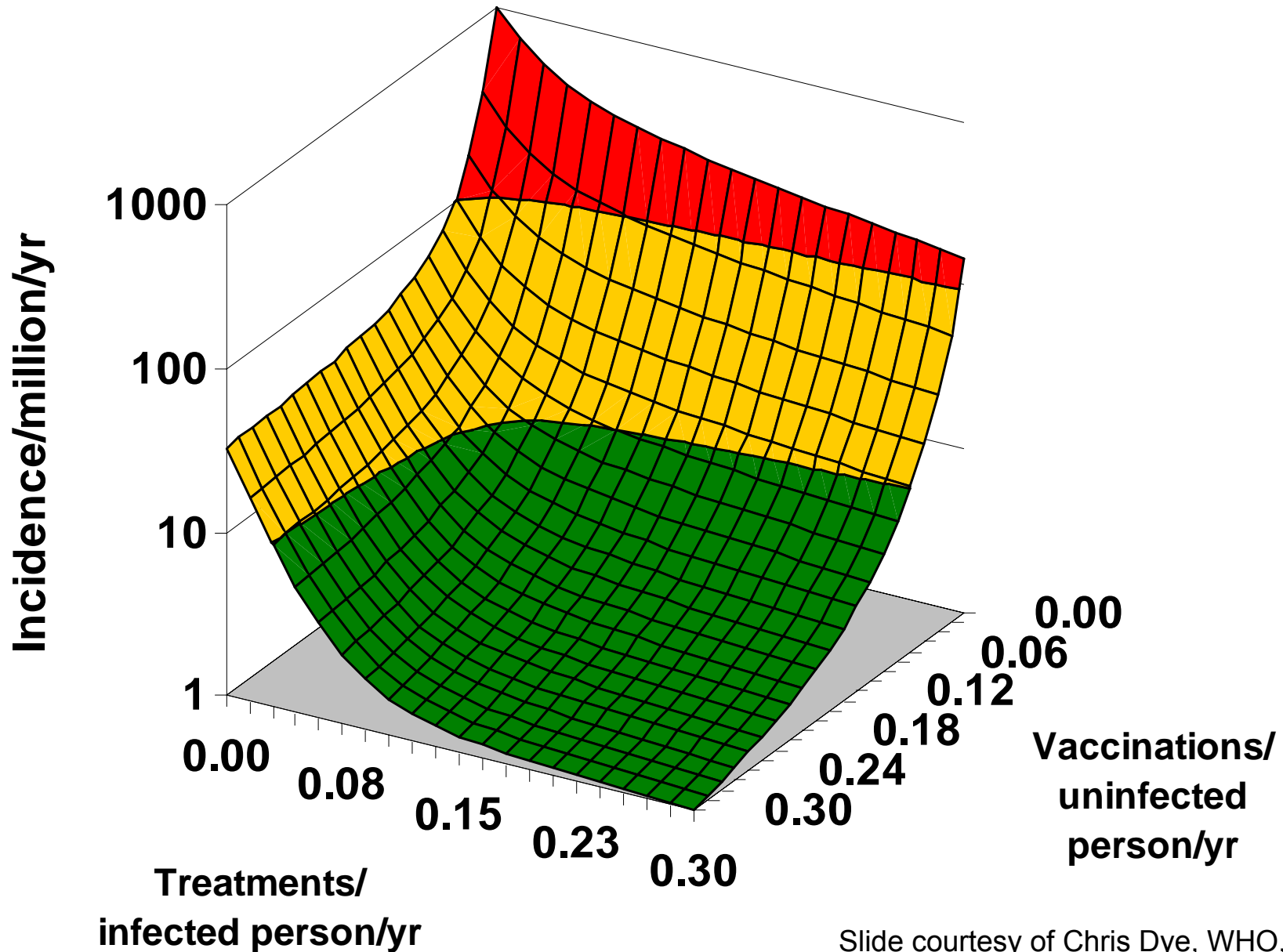
ELIMINATING TB BY 2050

BY TREATING ACTIVE DISEASE AND LATENT INFECTION



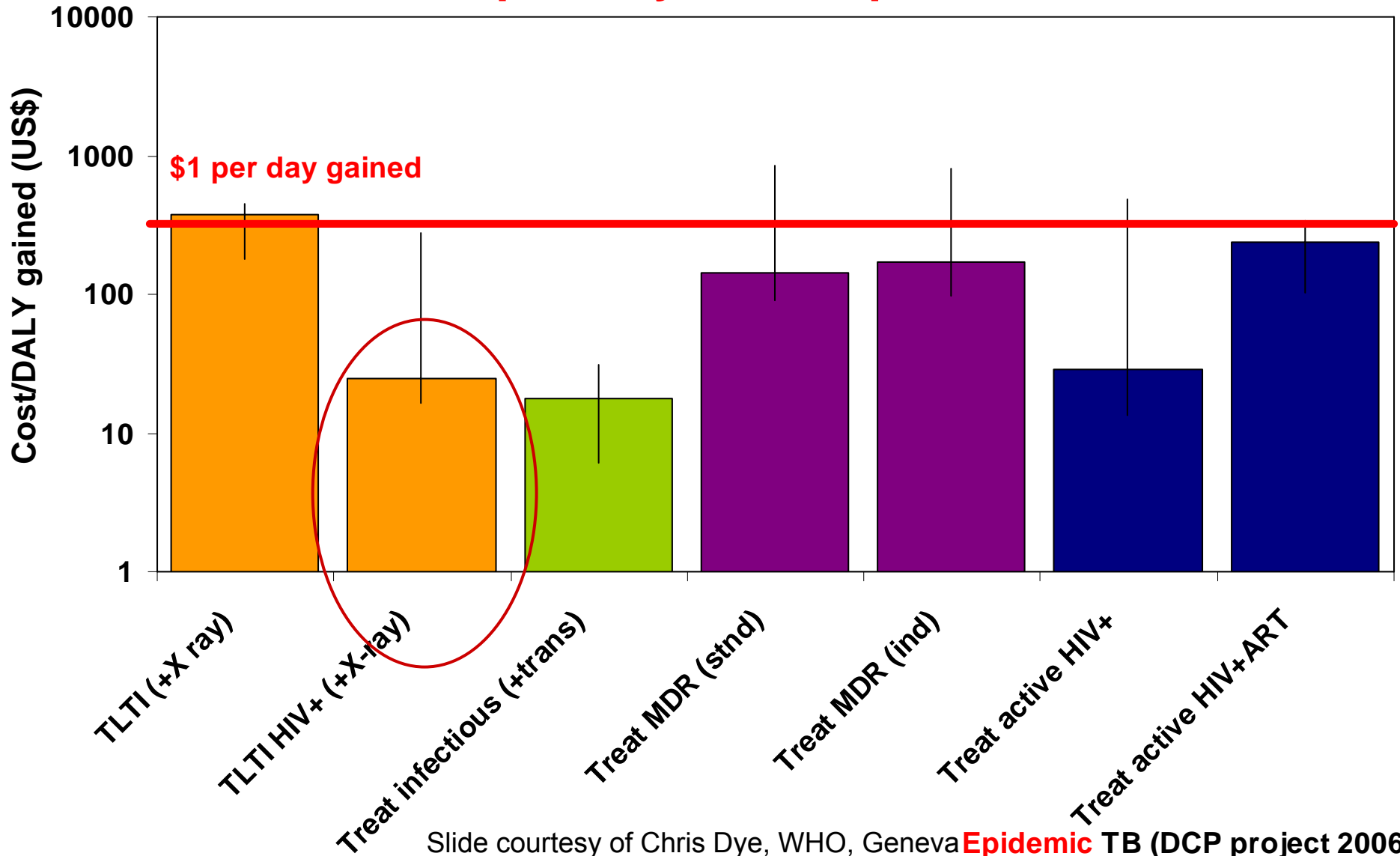
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ELIMINATING TB BY 2050 BY TREATING LATENT INFECTION AND VACCINATION



Slide courtesy of Chris Dye, WHO, Geneva

TLTI more cost-effective during epidemics especially for HIV-positives



Slide courtesy of Chris Dye, WHO, Geneva **Epidemic TB** (DCP project 2006)

Problems in diagnosing LTBI

The TST is inadequate in HIV-infected

- However, any systematic attempt to broaden the screening for LTBI in high-endemicity settings (whether in HIV-infected only or more generally) have been hampered by many well-known limitations of the TST¹ including low specificity, high rates of non-returns for reading and low sensitivity.
- Low sensitivity of the TST is a critical issue
 - HIV is a special case due to the extent and singularity of the immunosuppression and TST sensitivity falls dramatically (to 30-75% depending cutoff and stage of disease) in the HIV-infected^{2,3,4}
 - The falling sensitivity is allied to increasing rates of anergy; both phenomena are related significantly to CD4 count⁴ with false negative rates of between 30% (CD4 count >500/ μ l) rising to 100% with CD4 count <200/ μ l^{5,6}
- Attempts to overcome the falling sensitivity of the TST with HIV have been made by reducing the cut-off
 - But the success of this strategy relies on anergy being a gradual reduction in the delayed-type hypersensitivity response, rather than a mere “switch off” when the immune systems gets below a critical level of compromise
 - Emerging evidence⁷ suggests that it is a “switch-off” effect and therefore that reducing the TST cut-off didn’t significantly increase the sensitivity of the test as if a person was negative they were predominately anergic (0-2mm induration)

Introduction to the blood assays

- Two new blood tests for diagnosis of TB infection
 - T-SPOT.*TB* (Oxford Immunotec, Oxford, UK) – based on ELISPOT method
 - QuantiFERON-TB Gold (Cellestis, Melbourne, Australia) – based on ELISA method
- Both exploit the fact that TB infection induces a strong Th1 cellular immune response
 - But use different technologies to measure this response
 - Detection of a response is indicative of TB infection

	T-SPOT. <i>TB</i>	QuantiFERON-TB Gold	TST
Antigens	ESAT-6 and CFP10	ESAT-6 and CFP10	PPD
Positive internal control	Yes	Yes	No
Uniformity of methods and reagents	Yes	Yes	No [§]
Potential for boosting effect in repeated tests	No	No	Yes
Need for return visit	No	No	Yes
Time required for results	16–20 h*	16–24 h [‡]	48–72 h
Setting of test	<i>In vitro</i>	<i>In vitro</i>	<i>In vivo</i>
Interpretation of test	Objective (instrument based)	Objective (instrument based)	Subjective (operator-based)
Readout units	IFN- γ spot-forming cells	International units of IFN- γ	Millimeters of induration
Technological platform	ELISpot	ELISA	NA
Test's substrate	PBMC	Whole blood	NA
Outcome measure	Number of IFN- γ -producing T cells	Serum concentration of IFN- γ produced by T cells	NA
Readout system	Enumeration of spots by naked eye, magnifying lens, or automated counter [†]	Measurement of optical density values using an automated reader	Palpable induration

Taken from: Richeldi L *AJRCCM* 2006 174;736-742

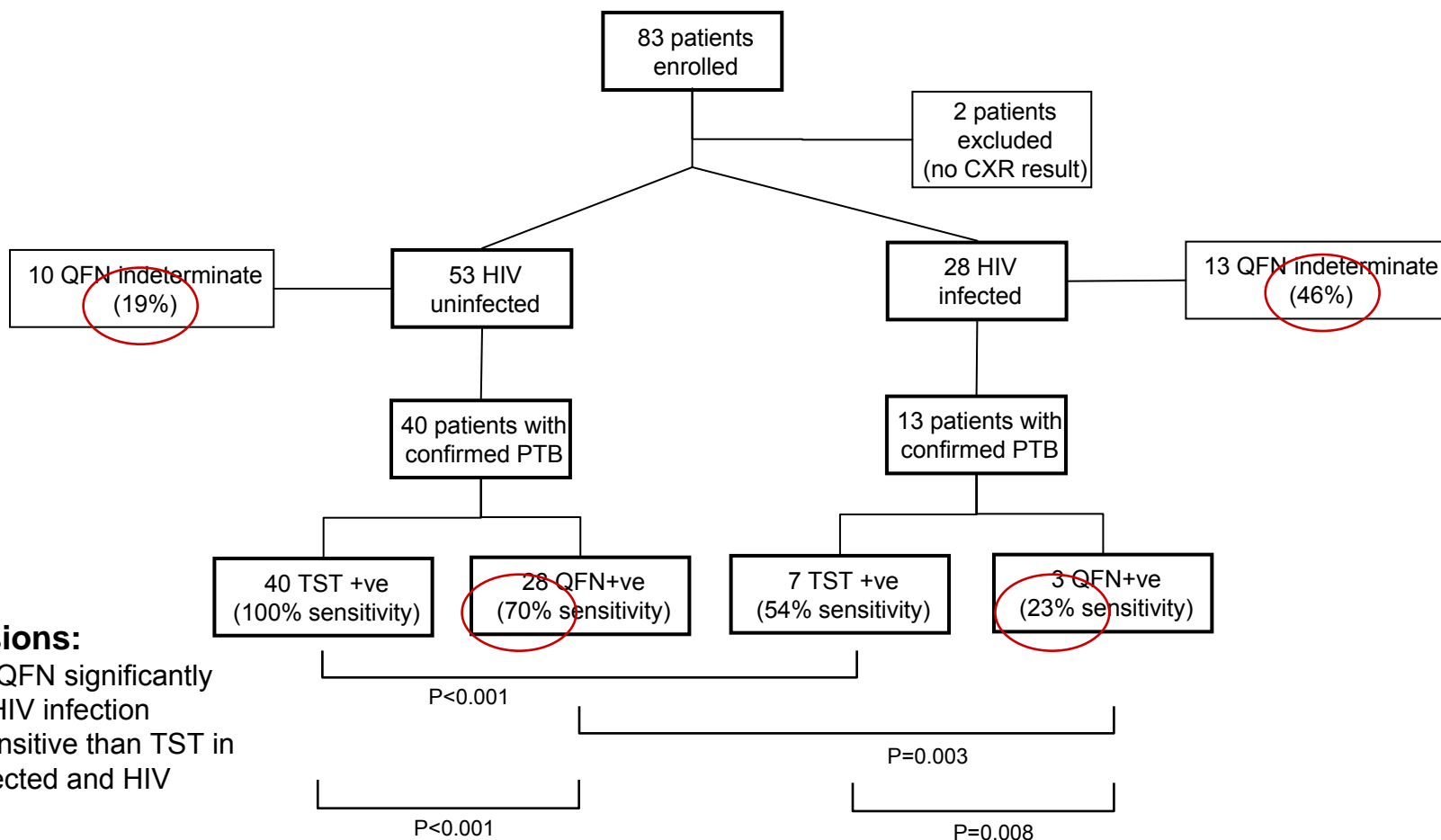
New blood tests

Potential advantages in HIV-infected & high endemicity

- High specificity
 - Both tests use the antigens ESAT-6 and CFP-10, which are not present in the BCG vaccine not in most non-Tuberculous Mycobacteria. This should avoid false-positive results obtained with the TST
 - ESAT-6 and CFP-10 are present in *M. marinum*, *M. szulgai*, *M.gordonae* & *M.kansasii* and positive results in patients infected with these mycobacteria are expected^{1,2}. In addition homologues of these antigens have been found in *M.leprae* and the performance of these tests have not been demonstrated in leprosy endemic populations.
- High sensitivity
 - These tests work on a different principle than delayed-type hypersensitivity and thus might be more sensitive than the TST; thereby picking up those patients at highest risk of progression to TB that are missed by the TST
 - It is an important question whether the new blood tests suffer from “anergy” like the TST too and whether there is a level of immunosuppression below which these tests also “switch off”
- A test that eliminates false positives and picks up those infected persons at highest risk of progression to TB should have higher predictive value of who will develop TB following a positive tests
 - This would radically shift the clinical and cost-effectiveness arguments towards more aggressive screening of at risk groups

QuantiFERON-TB Gold (In Tube)

Use of QFN-Gold in 83 adult TB patients in Tanzania



“The high proportion of indeterminate QFT and lack of sensitivity, particularly among the HIV infected patients, may limit its applicability in settings like Tanzania”

QuantiFERON-TB Gold (In Tube)

Use of QFN In Tube in TB cases in S.Africa

Study design:

- QFN In Tube & TST performed on 154 culture-confirmed TB cases in S. Africa.
- 26 HIV-infected, 16 HIV-negative, 112 HIV-unknown (but local epidemiology suggests 29% community HIV-seroprevalance and 55-61% in TB patients)

Study results:

- There was not a strong difference between sensitivity of either test in the HIV-confirmed subset (probably because the so many of the main study participants had undiagnosed HIV infection)
- QFN In Tube had 14.9% indeterminates in a population of high HIV seroprevalence
- TST was significantly more sensitive than QFN In Tube ($p=0.002$)

	QuantiFERON-TB Gold In Tube			
	Indeterminate	Positive	True sensitivity	Reported sensitivity
Culture-confirmed patients (n=154)	23 (14.9%)	100	64.9% (100/154)	76.3% (100/131)
Subset of confirmed HIV-infected (n=26)	5 (19.2%)	17	65.4% (17/26)	81.0% (17/21)
	Tuberculin Skin Test			
	No results	Positive	True sensitivity	Reported sensitivity
Culture-confirmed patients (n=154)	8	131	85.1% (131/154)	89.7% (131/146)
Subset of confirmed HIV-infected (n=26)	0	22	84.6% (22/26)	84.6% (22/26)

In 126 patients who had both TST and valid QFN results, TST sensitivity 90.5% (114/126) vs QFN sensitivity 76.2% (96/126) $p=0.002$ X^2 test

QuantiFERON-TB Gold (/In Tube)

Use of QFN In Tube in HIV patients in Denmark

Study design:

- QFN In Tube (no TST) was performed prospectively on 590 HIV patients attending the Department of Infectious Diseases during routine quarterly check-up

Study results:

- There was a strong correlation between anergy (indeterminate results) and the IFN-gamma responses to the mitogen control with CD4 count
- High level of indeterminates in those with low CD4 counts (24% in CD4<100/ μ l)

Correlation between CD4 cell count and response to PHA

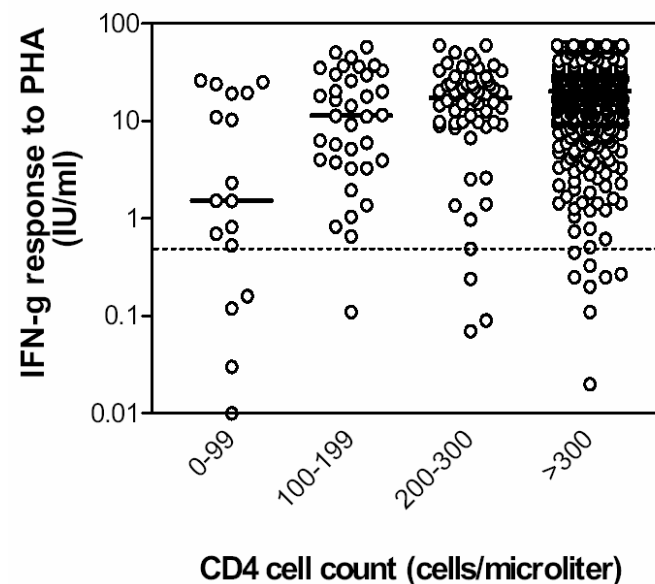


Table 3: Association between CD4 cell count and IFN- γ release in response to PHA.

CD4 cell counts ¹⁾	No of patients	Number and percentage of PHA <u>non-responders</u>	IFN- γ release (Median and 25–75 percentile)
<100	17	4 (24%)*	1,53 (0,34-19,49)#
100–199	37	1 (3%)	11,58 (3,88-30,11)
200–299	63	5 (8%)	16,78 (9,40-25,20)
>300	473	10 (2%)	20,35 (13,20–31,00)

T-SPOT.TB

Use of T-SPOT.TB in area of high TB-HIV prevalence in Zambia

Study design:

- Use of T-SPOT.TB* in area of high TB incidence (>500/100,000) and high HIV seroprevalence (23%); examination of performance in active and latent TB
- 50 Zambian sputum-smear positive TB patients (39 HIV-positive)
- 75 asymptomatic Zambian adults (21 HIV-positive). Population expected to have high-prevalence of LTBI. TST performed in 61/75 with 49 individuals returning

Study results:

- 0% had indeterminate T-SPOT results
- There was no significant difference in TB-antigen response between HIV+ and HIV-
- TST sensitivity (response rate) in LTBI declined significantly ($p=0.006$) between HIV- and HIV+, whereas T-SPOT.TB did not ($p=0.064$)
- T-SPOT.TB sensitivity fell slightly between HIV- and HIV+ TB patients but this was not statistically significant ($p=0.52$)

Response rates	Tuberculosis Patients		Asymptomatic adults	
	HIV- (n=11)	HIV+ (n=39)	HIV- (n=54)	HIV+ (n=21)
T-SPOT.TB	100% (11/11)	90% (35/39)	69% (37/54)	43% (9/21)
TST	-	-	80% (38/35)	36% (5/14)

T-SPOT.TB

Use of T-SPOT.TB in area of high TB-HIV prevalence in S.Africa

Study design:

- Prospective, blinded study of 293 child TB suspects with high rate of HIV seroprevalence (46%)
- Comparison of T-SPOT.TB and TST to eventual clinical diagnosis

Study results:

- T-SPOT.TB more sensitive than TST in confirmed TB (81% vs. 35% $p < 0.0001$)
- TST sensitivity significantly affected by young age, HIV and malnutrition ($p = 0.01, 0.002$ & 0.0003 respectively), whereas T-SPOT.TB was not ($p = 0.53, 0.12$ & 0.24 respectively)

	ELISPOT positive/ total tested by ELISPOT	Sensitivity of ELISPOT* (95% CI)	TST positive/total tested by TST	Sensitivity of TST* (95% CI)
Age				
>36 months	64/79	81% (71 to 89)	46/63	73% (60 to 83)
<36 months	46/54	85% (73 to 93)	27/53	51% (37 to 65)
p†		0.53		0.01
HIV				
Negative or not tested	88/103	85% (77 to 92)	64/91	70% (60 to 80)
Positive	22/30	73% (54 to 88)	9/25	36% (18 to 58)
p†		0.12		0.002
Z score				
>-2	61/71	86% (76 to 93)	48/63	76% (64 to 86)
<-2	46/59	78% (65 to 88)	22/50	44% (30 to 59)
p†		0.24		0.0003

*p values for difference between sensitivity of ELISPOT and sensitivity of TST in children who were aged <36 months, HIV positive, or very malnourished ($Z < -2$) were 0.001, 0.005, and 0.002, respectively. †p for difference in sensitivity of each test in children who were aged less than 36 months, HIV positive, or malnourished, versus children without these features (χ^2 test).

Table 4: Effect of age, HIV infection, and malnutrition on test sensitivity in children with confirmed or highly probable tuberculosis (n=133)

T-SPOT.TB

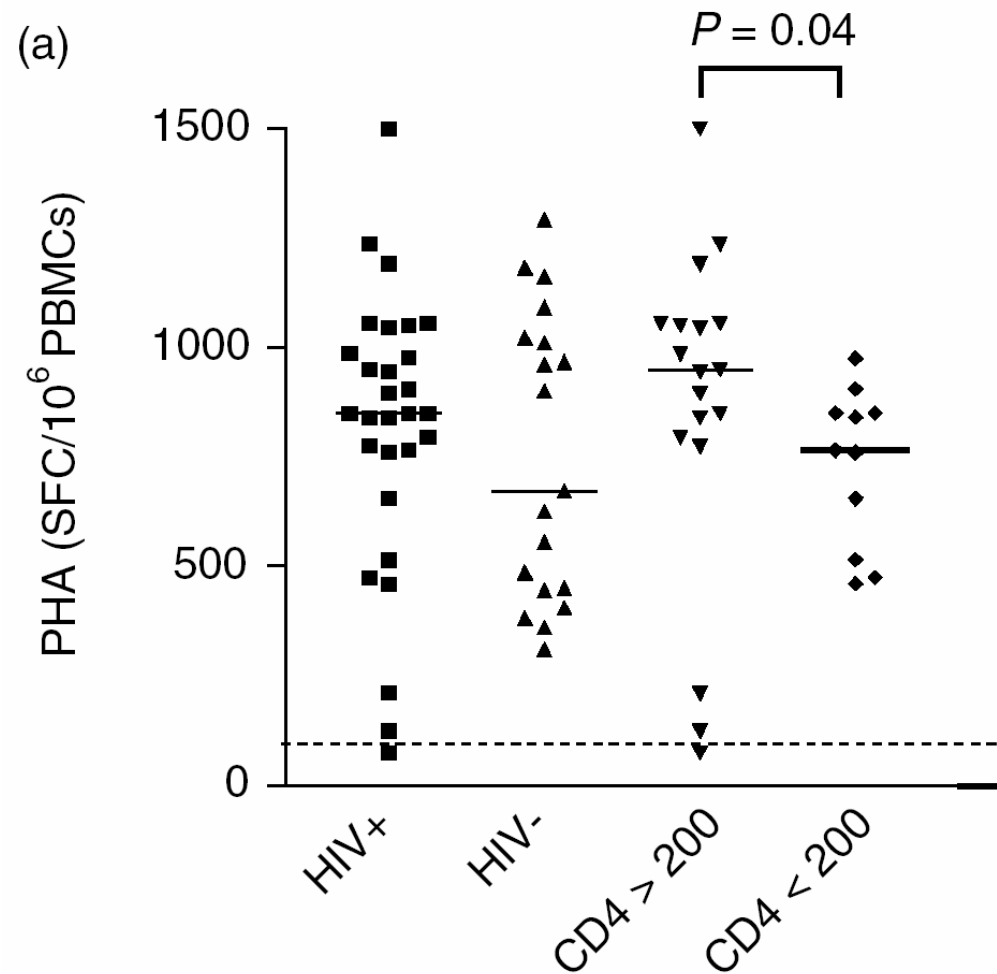
Use of T-SPOT.TB in HIV patients in UK

Study design:

- Comparison of mitogen-responses between 29 HIV-positive subjects and 19 HIV-negative subjects.

Study results:

- 3.0% (1/29) HIV-infected had indeterminate T-SPOT results. 0% of HIV-uninfected were indeterminate
- There was no significant difference in mitogen response between HIV-infected and HIV-uninfected
- There was a small difference in mitogen response between CD4 count <200 cells/ μ l and those >200 ; although the 1 indeterminate had CD4 >200 cells/ μ l



Blood tests in HIV-infected

Summary of evidence to date and comparison to TST

	TST	QFN-Gold	T-SPOT.TB
Clinical sensitivity <i>(active disease)</i>	30-75%	23% ¹ 65% ²	81% ³ 89% ⁴ 90% ⁵
Sensitivity vs. TST?*	n/a	Lower ^{1,2}	Higher ^{3,5}
Sensitivity affected by HIV infection?*	Yes	Yes ¹ No ²	No ^{3,4}
Anergy / Indeterminate rates	30-100%	46% ¹ 19% ² 3.4% ⁶	0.0% ³ 3.0% ⁴ 3.0% ⁷
Effect of CD4 count on response	Significant in all with HIV, but especially at CD4 counts <200/ μ l	Significant in those with CD4 counts <200/ μ l ⁵	Largely unaffected by CD4 count ^{6,7}

* At p<0.05 level in direct comparisons

1. Seshadri et al *IAS Conf HIV Pathog Treat 2005 Jul 24-27; 3rd: Abstract No. TuPe7.1C09*

2. Tsiouris et al *J Clin Micro 2006 44;8:2844-2850*

4. Kelleher et al. *Data presented at IUATLD 2006*

6. Brock et al *Respiratory Research 2006 7;56*

3. Chapman et al *AIDS 16;2285-2293*

5. Liebeschuetz et al *Lancet 2004 364;2196-2036*

7. Dheda et al *AIDS 2005 19;17:2038-2041*

Emerging evidence on blood tests in HIV-infected

Summary of evidence

- The specificity and other advantages of the IFN-gamma tests (e.g. no boosting, no return visit) are not really in dispute; but the key question is how badly they suffer from the same problems of sensitivity loss in the severely immunosuppressed:
 - Early evidence suggests that the tests suffer from this phenomenon less than the TST, but this may be technology specific (i.e. ELISPOT >> ELISA)
 - Of the two blood tests, T-SPOT.TB is the only one with possibly higher sensitivity than the TST; although study numbers are still small.
- Data on LTBI in HIV-infected individuals in high-endemicity settings is still extremely limited, and performance must largely be extrapolated from data in active TB and in low-endemicity settings.
- Further studies would be very helpful:
 - Larger-scale studies in high-endemicity settings with full testing for HIV and CD4 count
 - Understanding the predictive value of a positive blood test for eventual development of active TB
 - Head:head comparison of the two tests (and against the TST)
 - The possible cross-reaction of these tests to *M. leprae* should be investigated further

Diagnosing LTBI in the HIV-infected

Conclusions

- The identification of LTBI amongst HIV-infected in high-endemicity settings is extremely important; and likely to become ever more so as we move to better control, and ultimately elimination of TB.
- Any attempts to do this have, however, been hampered by the poor performance of the TST in the HIV-infected
- The new blood tests show promise for diagnosing LTBI more reliably in HIV-infected populations in high-endemicity settings

Diagnosing LTBI in the HIV-infected

Conclusions

- However, as well as more proof of their clinical performance, their widespread adoption in high-endemicity settings will also require a number of significant challenges to be overcome:
 - Cost of the tests
 - although it could be argued that even at current cost levels, if it could be proven that they were more sensitive and specific than TST, they would be cost-effective overall
 - Laboratory infrastructure
 - both tests are relatively complex and require equipment
 - Logistics
 - getting samples to an appropriately qualified lab within a sufficient time period
 - Gaining blood samples
 - although the TST suffers from high “non-return” rates, it is also not always possible to obtain a blood sample. For example, one recent study using one of these tests reported being unable to get blood samples in 17% of study participants¹
- Clearly the identification of infection is only half the battle. Without subsequent effective treatment of that infection the initial diagnosis is worthless. There are clearly significant challenges to the more widespread screening and preventative treatment, such as poor compliance to therapy². The experience of the provision of ARTs in Africa provides a salutary reminder of the challenges of administering widespread treatment³.

Conflict of interest statement

The author is an employee of Oxford Immunotec, which manufactures the T-SPOT. *TB* test

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