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Three Suitable Antigens for Delayed-Type Hypersensitivity Skin Testing in a Tropical Country like Thailand

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SUMMARY Delayed type hypersensitivity (DTH) skin test is a standard tool to assess *in vivo* cell-mediated immunity. Mantoux method using 4-5 common recalled antigens is recommended. However, not all antigens are widely available and appropriate antigens for tropical countries are not known. The objective of this study is to investigate what and how many antigens should be included in the DTH testing panel that suitable for Thailand and may be for this region. The DTH skin tests were done by Mantoux method in a double blinded fashion. Average induration size of ≥ 5 mm defined as a positive test. Antigens included purified protein derivative (PPD), *Candida albicans*, tetanus toxoid (TT), *Trichophyton mentagrophytes* and hepatitis B vaccine (HBV). The negative control was normal saline. Of 95 healthy subjects, all showed DTH positive to ≥ 1 antigen. The positivity to *C. albicans*, tetanus toxoid, PPD, *T. mentagrophytes*, and HBV was 92.6%, 83.2%, 82.1%, 50.5%, and 5.3%, respectively. When three antigens: PPD, TT and *C. albicans* were analyzed, 100% of subjects showed a positive response to ≥ 1 antigen and 96.8% showed a positive response to ≥ 2 antigens. When only PPD and TT were analyzed, 100% of subjects showed ≥ 1 antigen positive and 68.4% showed both antigens positive. *C. albicans* antigen at 1:100 was associated with a high incidence of fever (2/20) and large local reaction (7/20), 1:500 was found to be the optimal concentration. PPD, TT and *C. albicans* are suitable to be included in a DTH skin testing in a tropical country like Thailand. However, in a setting where *C. albicans* extract is not available, testing with only two antigens of PPD and tetanus toxoid may be an alternative, but with a lower sensitivity.

Delayed typed hypersensitivity (DTH) skin testing is a cost-effective *in vivo* tool to assess whether the patient has any clinical relevant T-cell defect. The main purpose of DTH skin testing is to evaluate a patient who clinically suggesting T-cell immunodeficiency. Multitest CMI system which has been widely used, is currently no longer manufactured because of the expense of maintaining potent, standardized testing antigens and the lack of consistent demand.[1] Previously recommended DTH testing by the Mantoux method using 3-5 antigens is therefore the mainstay *in vivo* cell-mediated immunity (CMI) evaluation.[1,2]

Results of DTH skin test vary with previous exposure to the antigens, age,[2-4] geography[5] and CMI function. Antigens that are recommended to be used in DTH skin test are *Candida albicans, Trichophyton mentagrophytes*, purified protein derivative (PPD), tetanus toxoid, mumps and diphtheria toxoid.[1-3,6,7] Experts suggested to use 4-5 antigens to evaluate *in vivo* CMI function by DTH skin test.[1,2] However, the precise number and specific types of antigens that should be used in DTH skin test remain controversial.

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Appropriate antigens for use in DTH skin testing in tropical countries such as Thailand are not known. Thus, to explore this issue, we performed DTH skin test with Mantoux method in healthy volunteers using 5 antigens: PPD (Thai Red Cross, PPD-TRC), *C. albicans* extract (Greer Laboratory Inc), *T. mentagrophytes* extract (Greer Laboratory Inc), tetanus toxoid (Pasteur Mérieux) and hepatitis B vaccine (Engerix™ B, GlaxoSmithKline). This study was to investigate what and how many antigens should be included in the DTH testing panel that suitable for Thailand and may be for this region.

The rationale of including hepatitis B vaccine is its wide availability in Thailand. In addition, CMI immune responses to hepatitis B virus have been observed.[8-11] Prevalence of previous hepatitis B virus infection with subsequent immunity in general population is 54% in Thailand.[12] Side effects of hepatitis B vaccine occurs occasionally; most commonly these are moderate soreness at the injection site in approximately 12% of vaccinees, mild fever in less than 2%, and other mild constitutional symptoms.[9] We therefore had included hepatitis B vaccine as an antigen to evaluate *in vivo* CMI function among Thai individuals.

METHODS

Population and study sample

Study subjects were Thai healthy volunteers age 15-60 years, with no previous history of serious infection (such as tuberculosis, severe pneumonia), not taking immunosuppressive medication (such as corticosteroids, cyclophosphamide) or no other secondary cause of CMI defect. Other exclusion criteria were DTH skin test within the previous 1-3 months,[1,2] major surgery in last 7 days,[13] autoimmune disease and pregnancy. These subjects would be excluded by history taking and questionnaires.

One Hundred volunteers participated in this study. The subjects were from the hospital based volunteers. However, 52% were non-health care personnel. Five of those screened were excluded: 2 had history of tuberculosis, 2 were taking systemic corticosteroids and one had carcinoma of the breast.

One limitation in this study regarding previous history of vaccination was that the majority of subjects did not have their medical records. Therefore, a working definition of BCG vaccination in this study is as following: "Definite evidence of BCG vaccination" demonstrated by when subjects had two vaccine scars, or one vaccine scar with a confirmed history of BCG vaccination before, or one vaccine scar and age younger than 24 years old since the official declaration of the eradication of naturally occurring smallpox in 1980,[14] when routine vaccination for smallpox had ended. "Probable evidence of BCG vaccination" would be applied if subjects had one vaccine scar without history of BCG vaccination, or no vaccine scar but with history of BCG vaccination. The study was approved by the Ethical Committee of the Faculty of Medicine, Chulalongkorn University, and written informed consent was obtained from the subjects.

DTH skin test

DTH skin tests were done by Mantoux method: 0.1 ml of each antigen was given by intradermal injection at the volar surface of the forearm, 3-4 cm apart between each antigen. To prevent bias when measuring the induration of the DTH responses, this study was performed in a double-blinded fashion: the investigator and the volunteers were blinded to all types of antigens and negative control from the injection steps to the measurement of the indurations.

Results of DTH skin test were measured at 48 hours by the ball-point pen method.[15] The maximum diameter and the perpendicular diameter were measured by drawing a line with a "medium" ball-point pen from a point 1-2 cm away from the margin of skin reaction, toward its center. When the ball point reached the margin of induration and definite resistance to further movement was noted, the pen was then lifted. This procedure was repeated again from the opposite side of reaction. DTH skin tests were considered positive if the average diameters of indurations were 5 mm or more. [1, 3]

Antigens and negative control

Five antigens were included in the study: PPD (Thai Red Cross, PPD-TRC, Bangkok, Thailand), *C. albicans* extract (Greer Laboratory Inc, North Carlolina, USA), *T. mentagrophytes* extract

(Greer Laboratory Inc, North Carlolina, USA), tetanus toxoid (Tetavax, Pasteur Mérieux, Lyon, France) and hepatitis B vaccine (Engerix^{TM} B, GlaxoSmith-Kline, Rixensart, Belgium). Negative control was normal saline. The concentration of PPD-TRC was 10 TU/0.1 ml that was equivalent to standard PPD-S from World Health Organization (5 TU/0.1 ml).[16] The concentration of other antigens were: *C. albicans* extract 1:500 w/v, *T. mentagrophytes* extract 1:20 w/v, tetanus toxoid 40 i.u./0.5 ml and hepatitis B vaccine 2 μg/0.1 ml.

Statistical analysis

A sample size of at least 95 volunteers was calculated from the following estimations: the positive DTH response of *C. albicans* extract was 63%,[17] maximum permissible error 10%, type I error was 5% and there was approximately 10% for loss to follow-up.

Positive DTH skin tests were considered positive if average induration size ≥ 5 mm.[1-3,6,18] To determine factors associated with the DTH positivity, univariate and multivariate analysis were performed using logistic modeling. Factors significantly associated with the responses to DTH skin test in the univariate analysis were examined in multivariate analysis by combining the relevant variables to control for the effect of each explanatory variable on the other variables studied. The size of the effect of each of the associated factors was measured by using the Odds ratio (OR) and 95% confidence interval (CI). All data were analyzed with SPSS 11.0.

RESULTS

Demographic characteristics

Demographic characteristics are summarized in Table 1. Of 95 subjects, 62 % were female with the mean age (SD) of 44.1 (10.1) years. Forty-eight percent were health care personnel, 9.5% had a history of contact tuberculosis (TB), 1.1% were chronic hepatitis B virus (HBV) carriers. In relation to previous vaccination: 33.7% and 61.1% had history of definite and possible BCG vaccination, respectively. Previous history of vaccination with tetanus and HBV vaccines was 78.9% and 15.8%, respectively. In subjects who had history of tetanus vaccination (n

Table 1 Demographic characteristics of 95 volunteers

Characteristics	N	%
Age (years), mean ± S.D. (range)	44.1 ± 10.1 (23-60)	
Sex: Female	59	62.1
Health care personnel	46	48.4
History of contact TB	9	9.5
History of hepatitis B carrier	1	1.1
Education level lower than secondary school	35	36.8
History of vaccination		
BCG vaccine -definite[†]	32	33.7
-probable[‡]	58	61.1
Tetanus vaccination	75	78.9
Hepatitis B vaccination	15	15.8
History of fungal infection		
Oral or vaginal candidiasis	10	10.5
***Dermatophyte* infection**[§]	23	24.2

[†]Definite evidence of BCG vaccination defined by when subjects had two vaccination scars, or one vaccine scar with history of BCG vaccination, or one vaccination scar and age less than 24 years.[14]
[‡]Probable evidence of BCG vaccination would be applied if subjects had one vaccine scar without history of BCG vaccination, or no vaccine scar but had history of BCG vaccination.
[§]Included history of fungal infection of nail, trunk, face, interdigital, axilla and inframammary area

= 72), 69.4% had the last vaccination more than 5 years ago. In subjects who had history of hepatitis B vaccination (n = 15), 80% had the last vaccination more than 5 years ago. Approximately one-third of the subjects reported concomitant medication use which included: antihistamines, NSAIDs and anti-hypertensive treatments.

DTH skin positive results

DTH skin tests were measured at 47.4 hours (SD = 3.8; range 43.5-74.7 hours). All subjects showed positive response to at least one antigen and showed negative to the negative control (normal saline solution). Eighty percent of subjects (n = 76) responded to at least 3 antigens. Positive DTH skin test against *C. albicans* extract was 92.6% which was the highest of any antigen tested. The positive responses to tetanus toxoid, PPD, *T. mentagrophytes* extract, and to hepatitis B vaccine were 83.2%, 82.1%, 50.5%, and 5.3%, respectively (Fig. 1).

Among subjects who showed positive DTH skin tests, the average diameters of induration skin reaction are presented in Fig. 2. The median diameters of PPD, Candida, Tricophyton, tetanus, hepatitis B vaccine was: 12.5, 17.8, 29, 14 and 12 mm, respectively. Twenty-seven subjects had an induration size reaction to PPD of more than 15 mm; 12 of these had a history of contact tuberculosis (TB) and/or were health care personnel. All of them had been recommended for chest X-ray evaluation. None of 19 who had the chest X-ray, showed features of pulmonary TB, and none had chosen to take isoniazid chemoprophylaxis.

To see if it was possible to minimize the number of antigens for DTH skin test screening, we calculated that 3 antigens compose of *C. albicans* extract**,** PPD and tetanus toxoid extract has a high positive response rate i.e. 100% showed positive reaction to at least 1 antigen whereas 96.8% had positive reactions to at least 2 antigens (Fig. 3). When only PPD and tetanus toxoid extract were used, 100% showed ≥ 1 antigen positive and 68.4% showed both antigens positive.

Factors associated with the positive DTH skin testing

To determine factors associated with a positive DTH skin test response, univariate analysis was performed (Table 2). Volunteers younger than than 40 years were associated with positive tetanus toxoid testing (OR 8.70 [1.10-69.31], $p = 0.02$); history of tetanus vaccination was associated with tetanus DTH positivity (OR 3.40 [1.01- 11.49], $p = 0.02$); male and a history of dermatophyte skin infection were associated with positive tricophyton DTH skin test response (OR 11.39 [4.04-32.13], $p < 0.0001$, and OR

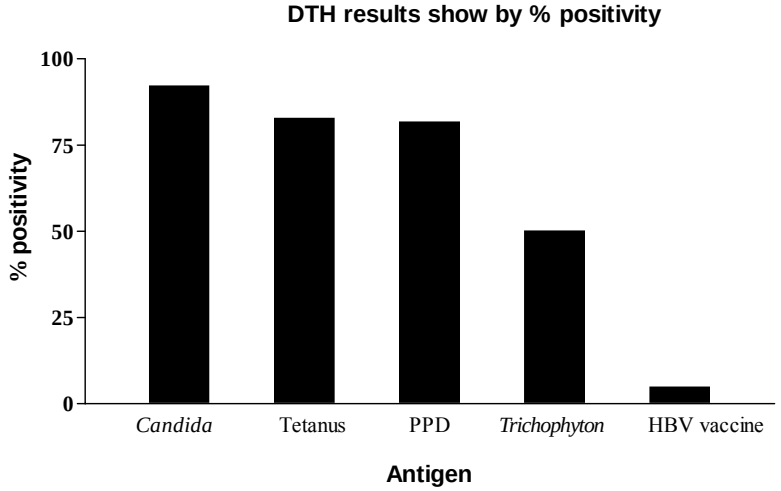

Fig. 1 The percentages of subjects with a positive response to various DTH antigen skin testing (N = 95).

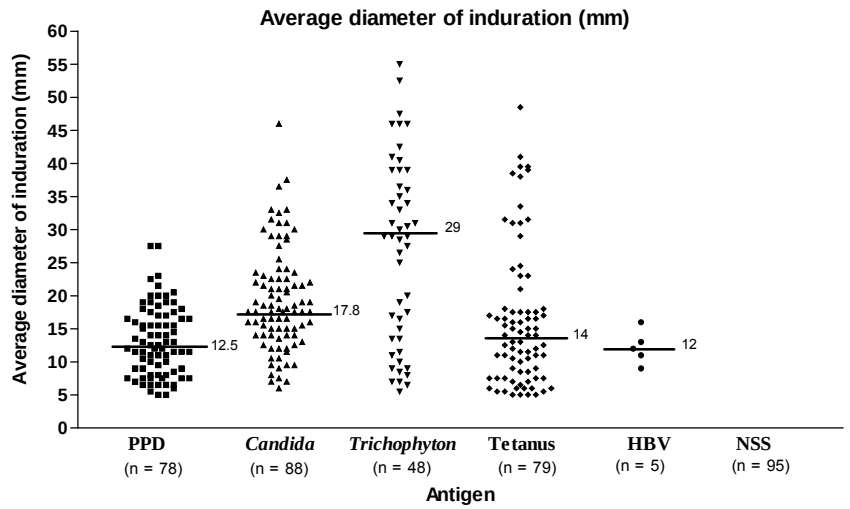

Fig. 2 Average diameters of the cutaneous indurations of individuals with positive DTH skin testing against the individual antigens (the horizontal line represent median value).

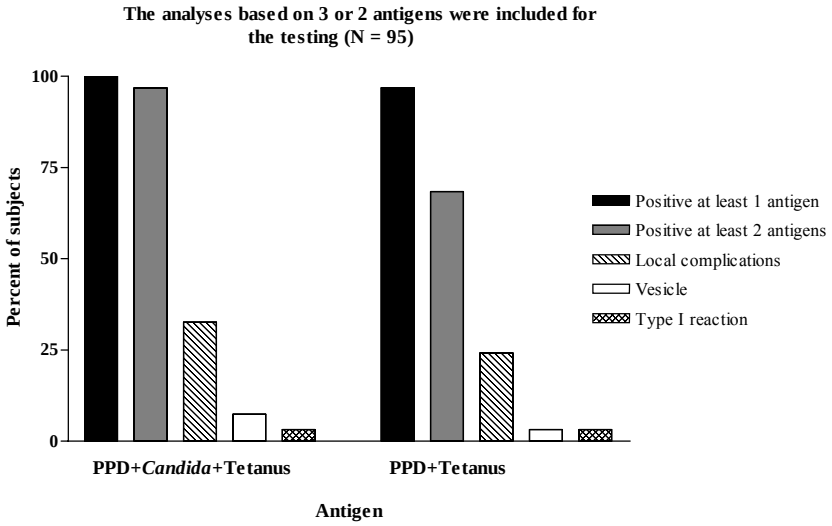

Fig. 3 The estimated percentages of the DTH positivity and adverse effects in a scenario when only 3 or 2 antigens were included in the DTH skin testing panel.

2.86 [1.05-7.79], $p = 0.04$, respectively); health care personnel was associated with PPD DTH positivity (OR 5.75 [1.52 – 21.74], $p < 0.01$); and the history of oral and/or vaginal candidiasis was associated with positive candidial DTH response (OR 0.12 [0.02 – 0.62], $p = 0.02$).

All of these significant unadjusted associated factors were then analyzed by binary regression analysis. The multivariate analysis results were summarized in Table 3. After the adjustment, factors that remained significantly associated with positive DTH responses were health care personnel (for PPD, OR= 4.65 [95% CI = 1.11-18.73], $p = 0.04$), male gender (males had more reaction to *T. mentagrophytes* extract (OR = 10.42 [95% CI = 3.57-30.30)], $p < 0.0001$), and history of tetanus vaccination (OR=3.87 [95% CI = 1.19-12.57]. $p = 0.02$).

Table 2 Factors associated with the responses to DTH skin test by Univariate analysis (N = 95, Unadjusted OR [95%CI])

	PPD	*Candida*	*Trichophyton*	Tetanus	Hepatitis B vaccine
No. of postive tests/total (%)	78/95 (82.1%)	88/95 (92.6%)	48/95 (50.5%)	79/95 (83.2%)	5/95 (5.3%)
Age 40 years or lower	0.82 (0.27-2.46)	0.59 (0.12-2.82)	0.80 (0.34-1.90)	8.70 (1.10-69.31)*	1.48 (0.23-9.43)
Male sex	1.15 (0.38-3.42)	1.57 (0.29-8.57)	11.39 (4.04-32.13)‡	0.746 (0.25-2.22)	0.39 (0.04-3.66)
Education level lower than secondary school	0.33 (0.11-0.97)*	1.13 (1.03-1.24)*	2.21 (0.94-5.21)	0.71 (0.24-2.10)	0.00 (0.00-1.97)
Health care personnel	5.75 (1.52-21.74)†	0.35 (0.06-1.89)	0.68 (0.31-1.54)	3.40 (1.01-11.49)	1.64 (0.26-10.30)
History of contact TB	0.74 (0.14-3.92)				
History of BCG vaccination	1.16 (0.12- 11.05)				
History of tetanus vaccination				3.95 (1.25-12.51)*	
History of hepatitis B vaccination					3.90 (0.59-25.63)
History of oral and/or vaginal candidiasis		0.12 (0.02-0.62)*			
History of *Dermatophyte* infection§			2.86 (1.05-7.79)*		

§Included history of fungal infection at nail, trunk, face, interdigital, axilla and inframammary area
*Statistically significant, $p < 0.05$, †Statistically significant, $p < 0.01$, ‡Statistically significant, $p < 0.0001$

Table 3 Factors associated with the responses to DTH skin test (Binary regression analysis, N = 95)

Antigen	Factor	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*p* value
PPD	Education level lower than secondary school	0.33 (0.11 – 0.97)	0.58 (0.18 – 1.87)	0.36
	Health care personnel	5.75 (1.52 – 21.74)	4.65 (1.11 – 18.73)	0.04*
***C. albicans* extract**	Education level lower than secondary school	1.13 (1.03 – 1.24)	2.69 (0.29 – 25.21)	0.85
	History of oral and/or vaginal candidiasis	0.12 (0.02 – 0.62)	4.93 (0.96 – 26.85)	0.07
Tetanus toxoid	Age 40 years or lower	8.70 (1.10 – 69.31)	1.21 (0.77 – 1.92)	0.42
	History of tetanus vaccination	3.40 (1.01 – 11.49)	3.87 (1.19 – 12.57)	0.02*
***T. mentagrophytes* extract**	Male	11.39 (4.04 – 32.13)	10.42 (3.57 – 30.30)	< 0.0001*
	History of *Dermatophyte* infection†	2.86 (1.05 – 7.79)	1.48 (0.45 – 4.83)	0.52

†Included history of fungal infection at nail, trunk, face, interdigital, axilla and inframamary area.
*Statistically significant, OR, Odds ratios; CI, confidence intervals.

Side effects related to DTH skin testing

Candida albican *extract of 1:100 w/v was associated with a higher incidence of side effects*

Of interest, when *Candida albicans* at of 1:100 w/v was initially included within the total of 6 double-blinded antigens (including the negative control- normal saline), among the first 20 subjects, 2 (10%) was associated with high fever and 7 (35%) had shown a large induration with tenderness to *C. albicans* antigen (the code was unblinded by an independent physician). To minimize these adverse effects, *C. albican*s extract was further diluted from previously recommended of 1:100 w/v[1] to 1:500 w/v. Of note, in 75 additional subjects, fever was decreased from 10% to 2.7% and the tenderness at induration site in response to *C. albican*s also de-

creased from 35% to 18%. However, the differences were not statistically significant. More importantly, there was no significant difference in the positivity results to the *C. albican*s DTH skin tests between these 2 concentrations (18/20 or 90% versus 70/75 or 93%, respectively).

Overall adverse effects

Adverse effects in this study included: fever, urticaria, moderate-to-severe local tenderness (required analgesic and/or 3 days course of 0.25-0.5 mg/kg/day of oral prednisone plus local corticosteroid cream application). Overall, adverse effects after the DTH skin testing with those 6 antigens occurred in 40% of 95 subjects: 37% with moderate-to-severe local tenderness, 7.4% fever and 4.2% urticaria (local urticaria 3 cases, generalized urticaria 1 case).

Type I hypersensitivity reaction (evidenced by wheal and flare reaction at the injection site and/or systemically) was found in 4.2% of subjects (n = 4). The causative antigens were: *C. albicans* extract (n = 3), *T. mentagrophytes* extract (n = 4) and tetanus toxoid (n = 3). All the reactions occurred within 15 minutes. Of note, one of them developed both local type I reaction and generalized urticaria (at the other forearm and trunk). In this individual, *T. mentagrophytes* extract was considered to be the cause.

When only 3 antigens were analyzed: *C. albicans* extract**,** PPD and tetanus toxoid extract, the local adverse effects was 32.6%. If we included only 2 antigens: PPD and tetanus toxoid extract, the local adverse effects was 24.2% (Fig. 3).

DISCUSSION

In this study we performed a double-blinded DTH skin testing to minimize technical and evaluation bias. Nevertheless, a population biased must be taken into account, as almost a half the study volunteers (48%) were health care personnel. Health care personnel have been reported to have been expose to some particular antigens more often than the general population, such as *Mycobacterium tuberculosis*[19-21] and hepatitis B virus.[9,22] We did not perform anti-human immunodeficiency virus (HIV) serology screening in this study; therefore the other possible confounding factor is secondary CMI deficiency due to AIDS. However, based on the recent UNAIDS/WHO estimation, in Thailand the HIV prevalence is approximately 1.5%. This figure may have a very small impact on the results and interpretation.[23]

Among 95 subjects tested, all showed positive response to at least one antigen. Eighty percent of subjects (n = 76) responded to at least 3 antigens. Positive DTH skin test against *C. albican*s extract was 92.6%, the highest positivity. The positive responses to tetanus toxoid, PPD, *T. mentagrophytes* extract, and to hepatitis B vaccine were 83.2%, 82.1%, 50.5%, and 5.3%, respectively (Fig. 1)

Our results on PPD, *C. albican*s extract**,** tetanus toxoid and *T. mentagrophytes* extract compared to previous studies are summarized in Table 4.

Table 4 Comparison of DTH skin test responses of this study to other studies

Authors	Method	Population				% of positive DTH skin test			
		N	Country	Description	Age (years)	PPD	*Candida*	Tetanus	*Trichophyton*
Kniker WT, 1984[34]	Multitest	402	USA	Healthy	17-92	58.7	83	86	53.5
Frazer IH, 1985[39]	Multitest	110	Australia	Healthy	38.8	67	48	65	29
Corriel RN, 1985[40]	Multitest	448	USA	Healthy	10.9	30.6	58.3	94.9	19
Kniker WT, 1985[41]	Multitest	221	USA	Healthy	4.2			62	
Cainzos M, 1993[5]	Multitest	1,476	Spain	Healthy	49.6	76.6	58.1	37.3	13.2
Brown AE, 2000[24]	Mantoux	70	Thailand	HIV+, CD4 > 400	NA	32	91	71	27
This study	**Mantoux**	**95**	**Thailand**	**Healthy**	**44.1**	**82.1**	**92.6**	**83.2**	**50.5**

NA, not applicable.

There is only one report from Thailand, however this is an HIV infected study population.[24] When we compared the results of this latter study in HIV infected patients with CD4 > 400, the percent positivity of *C. albicans* extract and *T. mentagrophytes* extract are comparable (92.6 vs. 91%, 88.2 vs. 71%, our results vs. Brown et al's results respectively). Our results were comparable to some studies conducted in health volunteers (Table 4). However, the PPD response results were high in our study as well as in the study reported from Spain.[5] There are several possible factors that may contribute to these results, including differences in geographic distribution, study population, sample size and immunization background.

Interestingly, there are no data on HBV specific DTH skin testing in healthy volunteers. HBV specific-T cell responses has been studied mainly in chronic HBV infection,[25-27] however those measurements were *in vitro* not *in vivo*. Our observations showed a low number of HBV DTH responders (only 5.3%). Some possible explanations for this result include: suboptimal amount of antigen,[28] some individuals may be chronic HBV carriers,[29] HBV immunization preferentially induces humoral and cytotoxic T-lymphocytes (CTL) CD8+ responses rather than CD4-mediated DTH responses.[30] In our study, 2 µg hepatitis B virus surface antigen (HBsAg) was used, whereas another study reported multiple intradermal 5 µg recombinant HBV antigen immunization led to all of the 6 individuals showing HBV specific antibody and DTH responses.[28] This may support the inadequate dosing issue. Another study has shown that patients who were persistent HBV carriers had predominantly T-helper type 2 (Th2) profile,[29] a factor which may lead to poor DTH responses among chronic HBV carriers. In Thailand, the prevalence of positive hepatitis B surface antigen (HBsAg) in blood varies from 5.1 to 9.3%.[12,31,32] Therefore, some of our study volunteers may have been affected by these issues. Nevertheless, our results indicate that HBV vaccine antigen is not suitable to be included in the DTH skin testing panel.

In our study, multivariate analysis has showed that 3 factors were associated with positive DTH responses: health care personnel (for PPD, OR = 4.65 [95% CI = 1.11 – 18.73], $p = 0.04$), male gender (males had more reaction to *T. mentagrophytes* extract (OR = 10.42 [95% CI = 3.57-30.30]), $p < 0.0001$), and history of tetanus vaccination (OR=3.87 [95% CI= 1.19 – 12.57]. $p = 0.02$). A high conversion rate of PPD skin test from negative to positive among the nurse students as of 95% has been reported by Heimbeck.[19,33] In this study, male volunteers had significantly more positive skin tests to *T. mentagrophytes* extract than female, however this finding was different from previous studies. For example, Kniker *et al.*[34] and Cainzos *et al.*[5] showed no gender specific differences in the DTH responses to *T. mentagrophytes* extract. The ethnic and geographic differences as well as sample size may have played a major role on this discrepancy. Of note, in our study, males reported more history of cutaneous fungal infection than females (41.7% vs. 13.6%, $p = 0.002$). The higher prevalence of skin infection may have led to a higher DTH response rates in males.

The association of tetanus vaccination history and the positive DTH skin test has previously been observed. Assamongkol and coworkers[35] found that children aged 9-18 months who had 3 doses of tetanus vaccinations had more positive DTH skin tests than children aged 4 months who had only one tetanus vaccination ($p < 0.0001$). Kniker *et al.*[4] showed that DTH responses would increase tremendously during the second year of life and DTH responses to tetanus toxoid were increased from 33% in children aged 6-12 months to 78.8% in those aged between 1-2 years.

The overall incidence of adverse effects after the DTH skin testing with the 6 antigens in our study were 40% of 95 subjects: 37% with moderate-to-severe local tenderness, fever (7.4%) and 4.2% urticaria (local urticaria, 3 cases, generalized urticaria, 1 case). In relation to type I hypersensitivity reactions; 3 volunteers showed a type I reaction (urticaria) to all 3 antigens; *T. mentagrophytes* extract, *C. albican* extract and tetanus toxoid. One volunteer had a local and systemic urticaria to *T. mentagrophytes* extract. This reaction has been reported in *T. mentagrophytes* extract and tetanus toxoid DTH skin testing.[36,37] All three antigens have been reported to induce type I hypersensitivity reactions. There is a speculation that a positive type I hypersensitivity reaction against one antigen may lead to a transient DTH anergy to that particular antigen.[1] This is not the case in our study since almost all of our study

subjects who developed type I hypersensitivity reaction also had positive DTH responses to *T. mentagrophytes* extract (3/4), *C. albicans* extract (2/3), and tetanus toxoid (3/3). Sorensen and Jones[38] has reported similar findings.

In reality, DTH skin testing is not routinely performed in most hospitals and not all antigens will be available. The practical question that clinical immunologists have been asked is what antigens should be included and how many of them are needed. To address this question, we have found that if 3 antigen are used (PPD, TT and *C. albicans)*, the chance to finding a positive DTH skin test to at least 2 antigens is approximately 97%, and to at least 1 antigen is 100%. We therefore suggest that PPD, TT and *C. albicans* are suitable to be included in a DTH skin testing in a tropical country like Thailand. When only two antigens were considered (PPD and TT), 100% showed ≥ 1 antigen positive and 68.4% showed both antigens positive.

We conclude that the appropriate antigens for DTH skin test are PPD, tetanus toxoid, *C. albicans* extract and *T. mentagrophytes* extract. In a tropical country like Thailand, at least 3 antigens: PPD, tetanus toxoid, and *C. albicans* should be recommended for a standard DTH skin testing. This set of antigens will cover 100 % healthy individuals with intact CMI status. However, in a setting where *C. albicans* extract is not available, testing with only two antigens of PPD and tetanus toxoid may be an alternative, but with a lower sensitivity.

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