

## Influence of vial size on the results of the tuberculin test\*

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**Background:** Tuberculin purified protein derivative is stored in vials of various sizes. Its adsorption to the vial can influence the results of tuberculin tests.

**Objective:** To evaluate the effect of vial size on the results obtained in tuberculin tests.

**Methods:** Sixty-four inpatients with active tuberculosis were submitted to two simultaneous tuberculin tests using the Mantoux technique. Patients were randomly allocated to receive two 0.1-ml injections, either one in the right forearm from a 1.5-ml vial and one in the left forearm from a 5-ml vial or vice versa. Induration was determined in a blinded fashion by a single, previously trained observer. Right arm-left arm differences of 2 mm or less were considered concordant results.

**Results:** Twenty-one patients presented no induration and were excluded from analysis. Among the 42 remaining patients, mean induration diameters obtained in tuberculin tests using the larger vials were greater than those obtained in tests using the smaller vials. Concordance was achieved in 40.5% (17/42). The difference was negative (large-vial indurations smaller than small-vial indurations) in 16.7% (7/42) and positive in 42.9% (18/42).

**Conclusions:** The size of the vial may influence tuberculin test results. Adsorption to the sides of the vial may explain this phenomenon. The authors caution others to be aware of the impact of these variations in epidemiological and operational studies.

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**Key words:** Tuberculin Skin Test. Tuberculin. Adsorption. PPD. Mantoux.

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## INTRODUCTION

Tuberculin purified protein derivative (PPD) is stored in vials of various sizes. The literature has registered that even siliconized vials may adsorb the active principle of the solution<sup>(1-3)</sup>. This phenomenon may lead to a decrease in the potency of the tuberculin and consequently interfere with tuberculin test (TT) results. In an article published in 1958, Waller et al. demonstrated that the intensity of the adsorption depends on the size of the vial, the power of the solution, the temperature at which it is stored, as well as the duration and extent of the contact between the solution and the inner surface of the vial<sup>(4)</sup>. Since 1958, the World Health Organization (WHO) has been using a stabilizing diluent called Tween 80 in order to decrease the adsorption of tuberculin to the vial.

The tuberculin used in Brazil is PPD RT-23, which is the biological equivalent of the standard PPD. The PPD RT-23 is produced by the Statens Serum Institut (SSI) in Copenhagen, Denmark. In the past, it was reconstituted and distributed by the WHO at no charge. In Brazil, the solution was then diluted in a public laboratory with Tween 80 and stored in 5-ml vials. However, in 1966, the partnership involving the WHO, the Pan American Health Organization (PAHO) and the Statens Serum Institut was dissolved. Until that time, this partnership had guaranteed the free distribution of the tuberculin to the PAHO member countries. Tuberculin (from the same source of production) then began to be sold to several countries, with private laboratories functioning as intermediaries (as wholesalers only, with no involvement in tuberculin production or storage). The tuberculin then became (and continues to be) available in vials of distinct sizes: the original 5-ml vial and a new 1.5-ml vial, containing approximately 50 and 15 doses, respectively.

The TT has been widely used in longitudinal studies evaluating the risk of infection by *Mycobacterium tuberculosis* and as a diagnostic test in high-risk situations, such as the monitoring of health professionals. In these studies, serial test results are frequently compared, and the execution technique should be standardized. There have been many studies reporting errors in interpreting TTs due to differences in application and reading techniques<sup>(5,6)</sup>.

Motivated by the need to standardize the activities of health professionals involved in operational projects developed in Rio de Janeiro, various training programs were conducted by the Academic Tuberculosis Program of the Thoracic Diseases Institute of the *Universidade Federal do Rio de Janeiro* (Rio de Janeiro Federal University). The present study was developed during one of these trainings and aimed to compare the results of TTs conducted with the same tuberculin, namely the PPD RT-23, stored in vials of various sizes.

## METHODS

A training program for health professionals from various institutions in the State of Rio de Janeiro was carried out in June 2002. The objective of this training was to standardize the activities of these professionals in the application and reading of TT results, as well as to evaluate and compare the results of readings obtained with tuberculin stored in vials of various sizes. On this occasion, 63 individuals with active tuberculosis (TB), all inpatients at the *Hospital Estadual Santa Maria* (Santa Maria State Hospital) in Rio de Janeiro, were submitted to two TTs (on the left and right forearms). The TTs were conducted simultaneously and only after written informed consent had been given.

The tests were conducted using the Mantoux technique, injecting 0.1 ml (2 IU) of PPD RT-23 in the middle third of each forearm using BD syringes (Plastipak, 1 ml, 13 x 3.8; Becton Dickinson, Franklin Lakes, NJ, USA) and disposable needles (PrecisionGlide, 27.5G 1/2; Becton Dickinson). Tuberculin had been stored in a refrigerator at a temperature ranging from 2°C to 8°C, protected from sunlight and in vials of two sizes: 5 ml (approximately 50 doses) and 1.5 ml (approximately 15 doses).

Patients were randomly allocated in pairs. The first patient in a pair received tuberculin from a large vial in the right forearm, the second received tuberculin from a small vial in the left forearm and so on consecutively, until there were no more pairs.

The reading was conducted 72 hours after the application, using the palpation method to determine the maximum transverse diameter of induration on each forearm, and the result was registered in millimeters. The reading was determined by a single observer blinded as to the

size of the vial used for the storage of the tuberculin applied. It is important to emphasize that the observer had been previously trained, and that there was a 97% intraobserver concordance.

Results were considered concordant when a difference of  $d \geq 2$  mm was found between the two readings. The means of both readings were compared, and the proportions of concordant and discordant cases were determined. Results were also classified as positive (induration  $\geq 10$  mm) and negative (induration  $< 10$  mm). The concordance between positive and negative results was calculated. Patients presenting no induration in both tests were excluded from the final analysis. The frequencies of concordant/discordant and positive/negative results were analyzed.

The study was approved by the Research Ethics Committee of the Rio de Janeiro Federal University Hospital *Universitário Clementino Fraga Filho* (Clementino Fraga Filho University Hospital) as part of the research project entitled "Risk of *M. tuberculosis* infection among medical students in the state of Rio de Janeiro".

TABLE 1

Frequency distribution of difference in induration from tuberculin tests conducted with large and small vials in 42 patients with active tuberculosis

Induration difference (small-large) mm	Frequency	Percentage
-5	2	7
-4	3	
-3	2	
-2	2	17
-1	3	
0	1	
1	3	
2	8	
3	5	18
4	3	
5	4	
6	2	
7	2	
8	1	
9	1	
TOTAL	42*	42*
		100%

\*21 pairs were excluded because they presented no induration on either arm.

## RESULTS

Twenty-one patients presented no induration in the readings of either forearm and were therefore excluded from analysis. The results of the 84 TTs administered to the remaining 42 patients and the respective differences are shown in Table 1.

The distribution of TT results by vial size is presented in Figure 1. When using a difference of  $d \geq 2$  mm between the right and left forearm readings as a criterion, we observed that concordance was achieved in only 40.5% (17/42), and that the difference was negative (large-vial indurations smaller than small-vial indurations) in 16.7% (7/42) and positive (large-vial indurations larger than small-vial indurations) in 42.9% (18/42). Among the 25 presenting discordance, 72% (18/25) were positive differences, and 28% (7/25) were negative differences.

## DISCUSSION

The TT, first described in the 19th century, is still the best diagnostic instrument for the detection of *M. tuberculosis* infection<sup>(7)</sup>. It is recommended for people who are at risk for infection, as well as for those at risk for progression of infection to active TB, who would benefit from treatment. Therefore, tuberculin testing has been recommended for health professionals, immunosuppressed individuals and those having come into contact with patients diagnosed with active TB<sup>(8)</sup>. Despite the existence of false-positive and false-negative results, this is the most appropriate and widely-available method for the diagnosis of latent TB infection, one of the three strategies of TB control, and has been adopted both in developed and developing countries<sup>(9,10)</sup>.

There are many factors that can interfere with TT results. Biological factors and conditions of transport and storage, as well as the techniques used in applying and reading the results, are all potential sources of error. In addition, false-negative results may be caused by immunodepression, and false-positive results may be caused by the high prevalence of nontuberculous mycobacteriosis and BCG vaccination<sup>(11)</sup>.

In this study, tuberculin stored in large vials was found to produce larger indurations than that stored in small vials. Figure 1 and Table 1 show

that most (72%) of the differences were positive (large-vial indurations larger than small-vial indurations). It is of note that, in this study, the syringes, needles, the application and reading technique, the observer, the patients and the tuberculin were the same in all tests. The only difference was the size of the vials. Biological variations greater than 6 mm have been accepted when serial tests are conducted with the objective of detecting recent TB infection<sup>(12)</sup>. Biological variations may explain part of the difference found between indurations, but no tendency for positive differences would be expected in this case.

One of the possible explanations for our results is that the relationship between tuberculin volume and internal surface of the vial available for adsorption is smaller in larger vials, therefore, interfering less with the potency of the tuberculin<sup>(4)</sup>.

In order to allow the comparison of results, it is recommended that tuberculin vials of equal size be used in transversal studies of *M. tuberculosis* infection prevalence and longitudinal studies of tuberculin test conversion. Waller et al. showed that

tuberculin potency decreases over time due to adsorption of the solution to the walls of the vial<sup>(4)</sup>. The findings of these authors reinforce those of the present study. Different correlations between internal layer and volume in vials of various sizes may explain this phenomenon. Nevertheless, we observed that the discordance between positive and negative results was not influenced by the size of the vial, suggesting that either type of vial may be used for a single TT. The sample was small, and subsequent studies should be carried out in order to confirm the findings.

Finally, we highlight the fact that, among the 63 patients with active TB studied, 21 (33%) presented negative tests with both tuberculins, and 7 (11%) had negative results, even with the large-vial tuberculin. It is known that false-negative TT results may be obtained in individuals known to have TB. Comorbidities, protein-caloric malnutrition and fever, as well as TB severity, have all been correlated to variations in TT results<sup>(13)</sup>. In addition, Tristão et al. recently demonstrated a correlation between tuberculosis and immunomodulation induced by helminth infections<sup>(14)</sup>.

	Large Vials (mm)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Small Vials (mm)	21																							
1																								
2									1															
3											1													
4						1		1																
5										1	1													
6						1						1												
7																								
8											1	1												
9						1																		
10								1	1	1			1				1		1					
11														1	1	1	1					1		
12										1					2		1							
13									1			1											1	
14															1		1	1						
15												2					1	1						
16											1							1					1	
17																	1					1		
18																						1		

Figure 1. Distribution of the tuberculin test results among patients with active tuberculosis, according to vial size. Grey cells show the limit where concordant results should be found. The concentration of readings to the right of the grey cells shows a trend toward larger indurations in the discordant results from larger vials.

In conclusion, the results of the present study should serve as a warning to researchers evaluating the risk of tuberculous infection in longitudinal studies, as well as for those who intend to make prevalence comparisons in transversal studies. Alterations in the size of indurations, rather than suggesting *M. tuberculosis* infection, may simply indicate reading and interpretation errors related to the use of tuberculins of different potencies resulting from variations in adsorption between vials of different sizes.

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