

Conservation of Purified Protein Derivative RT-23 Tuberculin

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OBJECTIVE: Recommendations that purified protein derivative (PPD) RT-23 tuberculin should not be kept and used more than 24 to 48 hours after opening are rarely complied with. The aim of this study was to determine whether using PPD RT-23 tuberculin from vials that had been open for a longer time could affect results of tuberculin tests and whether the solution could become contaminated.

METHODS: A prospective cross-sectional study was carried out. Two tuberculin tests were simultaneously administered, one in each forearm, to adult contacts of patients with active tuberculosis, one test using a recently opened vial of tuberculin (control) and the other using tuberculin that had been opened a week before (first phase) or a month before (second phase) (study tuberculin). Leftover tuberculin from several of the vials was cultured.

RESULTS: For the 127 patients in the first group (tuberculin opened 1 week), the mean (SD) diameter of the induration was 6.2 (6.9) mm for the study tuberculin and 6.3 (6.9) mm for the control ($P=.3$). For the 83 patients in the second group (tuberculin opened 1 month), the mean diameter of the induration was 5.5 (7.3) mm for the study tuberculin and 5.7 (7.3) mm for the control ($P=.5$). There were no differences between the number of positive tests found. None of the cultured tuberculins produced bacterial growth.

CONCLUSIONS: PPD RT-23 tuberculin does not appear to lose potency or sterility when vials have been opened for a week or a month.

Key words: Tuberculin. Tuberculin test. Conservation. Knowledge. Nursing.

Conservación de la tuberculina PPD RT-23

OBJETIVO: Aunque se recomienda que tras la apertura y utilización de los viales de tuberculina PPD RT-23 el producto restante no debe usarse pasadas 24-48 h, esto habitualmente no se cumple. El propósito de este estudio es evaluar si el mantenimiento de los frascos de tuberculina PPD RT-23 abiertos durante más tiempo de lo recomendado puede influir en los resultados de la prueba de la tuberculina o en la posibilidad de la contaminación microbiana del producto.

MÉTODOS: Estudio prospectivo transversal. Se realizaron de forma sincrónica 2 pruebas de la tuberculina en distintos antebrazos a contactos adultos de enfermos con tuberculosis activa, una con la tuberculina recién abierta (control) y otra con tuberculina de un frasco abierto una semana (en una primera fase) o un mes antes (en una segunda fase) (tuberculina estudio). Se cultivó la tuberculina sobrante de varios de los viales utilizados.

RESULTADOS: En los 127 pacientes del primer grupo (una semana), la media (\pm desviación estándar) del diámetro de la induración fue de $6,2 \pm 6,7$ mm con la tuberculina estudio y de $6,3 \pm 6,9$ mm con la control ($p = 0,3$). En los 81 pacientes del segundo grupo (un mes) fue de $5,5 \pm 7,3$ mm con la tuberculina estudio y de $5,7 \pm 7,3$ mm con la control ($p = 0,5$). No se observaron diferencias en la frecuencia de pruebas positivas. En ninguna de las tuberculinas cultivadas se obtuvo crecimiento bacteriano.

CONCLUSIONES: Parece que la tuberculina PPD RT-23 U2T no pierde potencia ni se contamina, aunque se mantengan abiertos los frascos durante una semana o un mes.

Palabras clave: Tuberculina. Prueba tuberculínica. Conservación. Conocimientos. Enfermería.

Introduction

Nearly 100 years after its introduction, the tuberculin test is still the most sensitive technique for the diagnosis of tuberculous infection and very important in identifying cases of tuberculosis itself.^{1,2} Incorrect administration or reading or inadequate storage can affect results.¹ 2TU

PPD RT-23 Tuberculin with Tween 80³ is recommended by the International Union Against Tuberculosis and Lung Disease (IUATLD)⁴ and the World Health Organization³ and is the tuberculin most frequently used in Spain. The above mentioned organizations,⁴ the manufacturers,⁵ and distributors in Spain⁶ recommend storage in optimum conditions of temperature and darkness and advise against keeping half empty vials for more than 24 to 48 hours, given the possibility of microbial contamination or loss of effectiveness. Most nursing staff, however, are unaware of these recommendations^{2,7} and we have not found studies that specifically analyze this aspect for the Evans PPD (RT-23) tuberculin (Celltech Pharma S.A., Madrid, Spain), which is the tuberculin used in our clinical context.

Part of this study was presented at the 36th National Congress of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) in Zaragoza, June, 2003.

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Manuscript received 28 October, 2003. Accepted for publication 1 January, 2004.

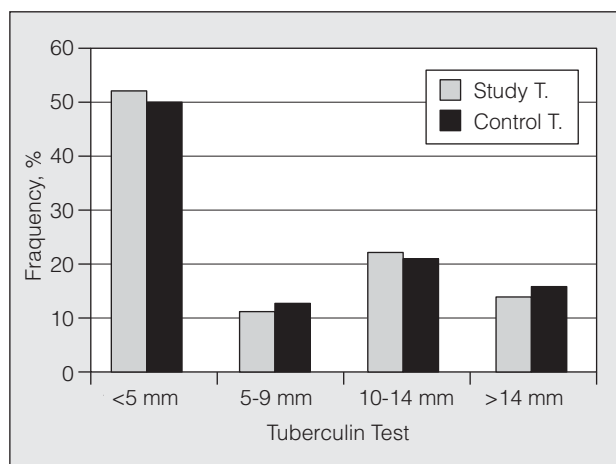


Figure 1. Results of the tuberculin test using control and 1-week study tuberculins. T indicates tuberculin.

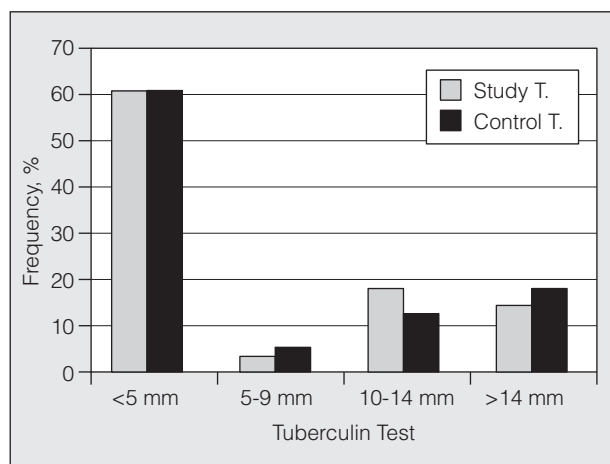


Figure 2. Results of the tuberculin test using control and 1-month study tuberculins. T indicates tuberculin.

The objective of this study was to examine whether keeping opened vials of PPD RT-23 tuberculin for longer than the recommended time could affect the results of the test or increase the risk of microbial contamination.

Methods

We carried out a prospective, cross-sectional study in which 2 Evans 2TU PPD RT-23 tuberculin tests were simultaneously administered, one in each forearm, to all subjects enrolled. Tuberculin from a recently opened vial (control tuberculin) was used in one of the tests and tuberculin from a vial from which several doses had been extracted and which had been opened a week before (in the first phase) or a month before (in the second phase) (study tuberculin) was used in the other test. The risk of contamination was also examined by performing microbiological cultures with the tuberculin left in vials that had been opened over a month before.

All tuberculin vials were kept under the recommended conditions of temperature and light exposure^{4,6} until immediately before their use. Study vials were selected from the same lot.

The patients enrolled in the study were adults who had been referred to our clinic because they had had contact with tuberculosis cases. Patients who had been administered tuberculosis vaccination, were over 65 years of age, or who were known to have some type of immunodepression (human immunodeficiency virus, corticosteroid or immunodepressant treatment, etc) were excluded from the study.

The tuberculin test was carried out by nurses trained in its administration and according to the IUATLD recommendations.⁴ Nurses injected 0.1 mL of 2TU PPD RT-23 of each tuberculin, choosing the side without regard to any particular criteria. The tuberculin test was read 72 hours later by another nurse experienced in this test and blind to which tuberculin had been injected into each forearm. The presence of vesiculation, necrosis, ulceration, or possible infection at the puncture point was also analyzed. All tests with induration greater or equal to 5 mm were considered positive in accordance with guidelines from the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR).⁸

Patients gave their verbal consent to participate in the study.

Statistical Analysis

The χ^2 test with Yates continuity correction was used to compare discrete variables, the Fisher exact test being used when values were expected to be less than 5. Normality of distribution of quantitative variables was analyzed using the Kolmogorov-Smirnov test, the paired Student *t* test was applied if distribution was normal and the Wilcoxon signed rank test was used if it was not. *P* values less than or equal to .05 were considered significant and means (SD) were calculated.

Calculations were carried out using the statistical program Statistical Package for the Social Sciences (SPSS), version 9.0 (Chicago, Illinois, USA).

Results

Two hundred eight patients were enrolled in the study, 127 in the group with week-old vials and 81 in the group of month-old vials. Of the 127 patients of the first group, 69% were men and the mean age was 36 (11.3), with a range of 15 to 65 years. Tuberculin test induration measured 6.2 (6.8) mm (range, 0-24 mm) for the study group (week-old vials) and 6.4 (6.9) mm (range, 0-27 mm) for the control tuberculin (*P*=.3). The rate of positive tests (≥ 5 mm) was 47.9% for the study tuberculin and 50.4% for the control tuberculin (*P*=.7). The only test that presented vesiculation was with control tuberculin (*P*=.9). Results for 4 diameters of induration are presented graphically in Figure 1. Three patients were considered negative with the study tuberculin and not with the control but differences were only 1 mm in 2 cases and 2 mm in 1 case.

Of the 81 patients of the second group, 79% were men and the mean age was 37.8 (13.3), with a range of 16 to 62 years. Tuberculin test induration measured 5.5 (7) mm (range, 0-26 mm) for the study tuberculin (month-old vials) and 5.7 (7.3) mm (range, 0-26 mm) for the control tuberculin (*P*=.5). Positive tests were recorded in 37.8% for both tuberculins. Results for 4 diameters of induration

are presented graphically in Figure 2. One patient presented a positive test for the study tuberculin and not the control tuberculin and another patient presented the opposite. Vesiculation was present in a total of 5 patients (6.2%) with the study tuberculin and a total of 5 with the control tuberculin (6.2%), 4 of the patients presenting vesiculation for both tuberculin tests.

Cultures were made of the leftover tuberculin from 12 randomly selected vials from which several doses had been extracted on different days and which had been kept at 4°C for a minimum of 1 month (68.6 [34.5] days; range, 34-155 days). No bacterial growth was obtained in any of them.

Discussion

The capacity of Evans 2TU PPD RT-23 vials is 1.5 mL, enough for approximately 10 to 12 doses. Each vial costs €14.07.

The IUATLD⁴ recommends that PPD RT-23 tuberculin should be used for only 2 days after the vial has been opened. A monograph published by Statens Serum Institute of Copenhagen,⁵ where the tuberculin is produced, recommends discarding the vials 24 hours after they have been opened in order to avoid bacterial contamination. According to the drug prospectus,⁶ half-empty vials should not be kept in case potency is reduced through adsorption. However, nurses—who are usually responsible for administering the test—are frequently unaware of these recommendations.² Córcoles et al,² in a recent study of 100 primary health care centers, found that 72.7% had open vials of tuberculin and no indication of the date they had been opened. In a questionnaire given to 203 nurses in our field, 76% were unaware of the above-mentioned recommendations.⁷

Implementation of these recommendations would result in discarding most of the product, particularly in clinics that do not regularly administer the test. This fact, together with lack of awareness of the recommendations, could lead to many staff using open vials that have exceeded the use-by date. The local distributors were unable to provide us with references on this question. Although there are references in the medical literature regarding keeping tuberculin PPD RT-23 test results,⁹ searching the MEDLINE database did not reveal studies examining loss of potency between 2 tuberculins of the same type and lot but that had been open for different lengths of time. Furthermore, our experience has suggested that potency remains the same and although we have been complying with the manufacturer's instructions in our clinic, we have not observed differences in the number of cases found or skin infections in patients who received the skin test in centers that do not fulfill the recommendations.

The results of the present study, despite its small size, seem to confirm that tuberculin PPD RT-23 does not lose potency in vials that have been open a week or even a month. Neither did we find bacterial contamination or local infections. Our results are consistent with the recommendations of other tuberculin manufacturers such as Turbesol (PPD-S, Aventis-Pasteur Inc. Swiftwater, USA). In the manufacturer's instructions for this tuberculin, much used in the USA, vials that have been open for more than 30 days are not recommended for use in case oxidation and degradation reduce potency.¹⁰

Despite finding a slight, nonsignificantly higher percentage of positive skin tests with the control tuberculin compared with the 1-week study tuberculin, no differences were observed with the 1-month study tuberculin. The higher tendency seen with the 1-week tuberculin might be attributable to the variability produced by administering skin tests in 2 separate places or to intra-rater reliability.¹¹

Larger studies with more patients could confirm these results and allow the manufacturer to change the instructions for storage, precipitating a reduction in costs, particularly in clinics that do not regularly administer the test.

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