INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 26, no. 3, 807-808 (2013)

LETTER TO THE EDITOR

IMPROVEMENT OF A RAPID SCREENING TEST FOR CHRONIC GRANULOMATOUS DISEASE

M. IACOBINI, M. DUSE, A. DI COSTE and L. BALDUCCI

Department of Pediatrics, Policlinico Umberto I, "Sapienza" University of Rome, Rome, Italy

Received April 4, 2013 – Accepted May 10, 2013

Diagnosis of CGD is made by demonstrating absent or markedly reduced oxidase activity in stimulated neutrophils. The screening test proposed is based upon the naked eye evaluation of the reduction of NBT on a solid surface. It seems to be a useful tool for rapid and inexpensive detection of CGD patients, especially for large-scale screening purposes. The test was carried out on forty-five subjects: two males affected by CGD, three female carriers and forty healthy donors. The test confirmed the results obtained with flow cytometric and NBT assays.

Diagnosis of Chronic Granulomatous Disease (CGD) can be made by counting nitroblue tetrazolium chloride (NBT)-reducing phagocytes as well as by Cytochrome c reduction (1-3) and flow cytometric assays (4). However, the requirements of large amounts of blood, of skilled technical help, and of a well-equipped laboratory render these tests unsuitable for screening and studying CGD patients.

The aim of our investigation was to promote a reproducible, inexpensive and rapid procedure. The screening test proposed is based upon the naked eye evaluation of the reduction of NBT on a solid surface (5). A glass or plastic plate is prepared, containing a solid substrate with the following composition: Kaolin (K7375, Sigma-Aldrich Co, Italy) 200 mg; NBT (N6876, Sigma-Aldrich Co, Italy): 0.2% solution in Phosphate Buffer Saline (PBS), calcium and magnesium free, pH 7,3, 2 ml and Agar (Bacteriological Agar, A5306, Sigma-Aldrich Co, Italy): 4% solution in distilled water, pH adjusted to 7,3 with NaOH normal solution, 2 ml. A homogeneous suspension of Kaolin in NBT solution is prepared in a beaker; the melted Agar, maintained at 70-80°C, is added. The suspension is thoroughly mixed by hand agitation, and the surface of the plate is covered with a thin layer of it. Let cool and solidify at room temperature. The plates, sealed with scotch tape, protected from light and stored at 4°C, maintain their efficiency for at least five months. A drop of fresh blood, obtained by fingerstick or venipuncture, is placed on the solid surface; heparinized blood can also be used. The plate is placed, in air, at 37°C for 30–60 min. During incubation, the phagocytes adhere to the agar surface and react, producing blue formazan. After incubation, blood is gently rinsed off with water. Also the adhering phagocytes are washed off, but the areas corresponding to them appear well delimited, stable and blue stained, due to NBT reduction. The test was performed by us on forty-five subjects: two males affected by CGD, three female carriers and forty healthy donors, aged 4-40 years. Normal subjects showed an evident blue staining, female carriers a very slight staining, while CGD patients no staining of the substrate (Fig. 1). The test confirmed the results obtained in 1993 by Iacobini et al. (3) with flow cytometric assays and NBT-densitometric kinetic

Key words: chronic granulomatous disease, screening, nitroblue tetrazolium chloride

Mailing address: Dr. Annalisa di Coste,
Department of Pediatrics,
Policlinico Umberto I, "Sapienza" University of Rome
Viale Regina Elena 324, 00161 Rome, Italy
Tel.: +39 06 49979308; +39 3299232524
Fax: +39 06 49979377
e-mail: dicoste.annalisa@gmail.com

0394-6320 (2013)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

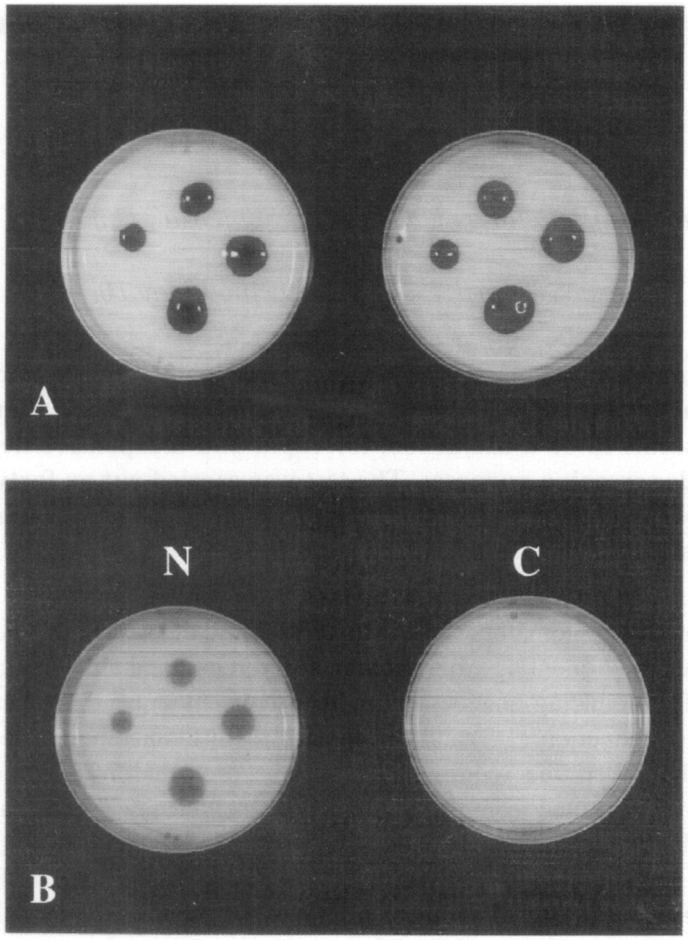

Fig. I. *Plate with blood drops before (A) and after (B) incubation and washing. N= Normal control: intense staining. C= CGD patient: no staining.*

test. The test proposed has not sufficient sensitivity for carrier detection, therefore more sensitive assays are necessary. It seems to be a useful tool for rapid and inexpensive detection of CGD patients, especially for large-scale screening purposes.

REFERENCES

1. Park BH, Fikrig SM, Smithwick EM. Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet 1968; 2:532-34.
2. Ochs HD, Igo RP. The NBT slide test: a simple screening method for detecting chronic granulomatous disease and female carriers. J Pediatr 1973; 83:77-82.
3. Iacobini M, Torre A, Palumbo G, Del Principe D, Digilio G, Balducci L. Study of chronic granulomatous disease by a nitroblue tetrazolium densitometric kinetic test: a new research method. Clinica Chimica Acta 221 1993; 197-202.
4. Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE, Brown MR, Anderson-Cohen M, and Fleisher TA. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the dihydrorhodamine 123 assay. J Allergy Clin Immunol 2003; 111:374-9.
5. Balducci L, Battaglia M, Midulla M. Prova diagnostica rapida per la malattia granulomatosa cronica (Rapid diagnostic test for chronic granulomatous disease). Boll Soc Ital Biol Sper 1975; 51:499-504.