

# Seroprevalence of ML Flow test in leprosy contacts from State of Minas Gerais, Brazil

## Soroprevalência do teste ML Flow em contatos de hanseníase de Minas Gerais

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### ABSTRACT

Leprosy is a public health problem in Brazil. Epidemiological control actions are based on the diagnosis and treatment of leprosy patients and household contact surveillance. Serological tests for leprosy could identify from among the contacts those at greater risk of developing leprosy in the future. ML Flow was administered to 2,840 household contacts of new leprosy cases diagnosed from October 2002 to March 2004, in State of Minas Gerais. ML Flow was positive in 20.5% of leprosy contacts, with high seropositivity among males (22.4%), individuals aged over 15 years-old (21.7%) and individuals in contact with multibacillary cases (23.9%). The chances of a household contact presenting a seropositive test was related to household contact with a multibacillary index case (OR=1.75), age over 15 years-old (OR=1.38) and male gender (OR=1.25). Follow-up of these contacts is necessary to evaluate the real role of seropositivity in the development of leprosy disease.

**Key-words:** Leprosy. Serology. Leprosy/epidemiology. Leprosy/prevention and control.

### RESUMO

A hanseníase é um problema de saúde pública no Brasil. As ações de controle estão baseadas no diagnóstico e tratamento dos indivíduos doentes e na vigilância de seus contatos. Os testes sorológicos permitem identificar, entre os contatos, aqueles com maior risco de desenvolver hanseníase. O ML Flow foi utilizado em 2.840 contatos intradomiciliares de casos novos de hanseníase, diagnosticados entre outubro de 2002 e março de 2004, em Minas Gerais. O ML Flow foi positivo em 20,5% dos contatos de hanseníase, sendo maior nos contatos do sexo masculino (22,4%), nos maiores de 15 anos (21,7%), nos contatos de doentes multibacilares (23,9%). A chance de um contato ser soropositivo foi maior se convivia com caso multibacilar (OR=1,75), idade superior a 15 anos (OR=1,38) e sexo masculino (OR=1,25). O acompanhamento desses contatos permitirá, no futuro, avaliar o risco que a soropositividade representa no desenvolvimento de hanseníase.

**Palavras-chaves:** Hanseníase. Sorologia. Hanseníase/epidemiologia. Hanseníase/prevenção e controle.

Leprosy is a chronic, infectious and granulomatous disease, which mainly affects the skin and peripheral nerves. It is a public health problem in Brazil, where 40 to 50,000 new cases occur per year.

Leprosy contacts are the population at greatest risk of developing leprosy. These are individuals who live together with leprosy patients. Household contacts that have lived with leprosy patients in the preceding five years must have priority, according to the national leprosy control program<sup>6</sup>. Household contacts of multibacillary leprosy patients present a 5 to 14-fold greater risk

of developing leprosy, while household contacts of paucibacillary leprosy patients present twice the risk than the general population<sup>27 16 21 32</sup>.

It is important to remember that leprosy diagnosis is based on clinical aspects. The development of serological tests has occurred in the last twenty years, but it is important to stress that none of these are diagnostic tests. The ML Flow test is an immunochromatographic flow test for the detection of IgM against PGL-I, for which results are obtained between 5 to 10 minutes, using whole blood or serum. Laboratory and refrigeration<sup>9</sup> are not necessary. The test is correlated with the presence of *Mycobacterium leprae* in the host<sup>9</sup> and patient positivity is associated with bacterial load. Reports indicate that serology shows greater sensitivity than skin smears and can be used to classify confirmed leprosy patients as multibacillary or paucibacillary, in addition to identifying contacts of leprosy patients with a greater risk of developing leprosy<sup>9 22 25</sup>.

One of the aims of leprosy control programs is to examine household contacts to enable early diagnosis and treatment. The possibility of using the ML Flow test in leprosy patients and

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household contacts in the public health services could be very useful in implementing this action.

The objective of this study was to verify the seropositivity of ML Flow in household contacts of new leprosy patients.

## MATERIAL AND METHODS

### Population

This is a descriptive and cross-sectional study which compared the results of the ML Flow test in household contacts of new leprosy cases detected in 14 health services of 13 municipalities in the State of Minas Gerais. The household contacts were examined, according to recommendations of the Ministry of Health<sup>5</sup>, and submitted to the ML Flow test, after signing the free and informed consent form. Household contacts of new leprosy cases detected during the period of October 2002 to March 2004 were included. The contacts were classified according to the index case in contacts of multibacillary or paucibacillary patients. Seropositive contacts should be followed-up by the health services every six months for four years.

### Serology

The ML Flow test is composed of a semisynthetic antigen and a natural trisaccharide linked to bovine serum albumin (NT-P-BSA)<sup>9</sup>. The test was performed with whole blood taken via finger prick and the result was read 5 min later. The test scored positive when red staining of the test line was observed and scored negative when no staining was observed. In both cases, the control line must be observed to guarantee the validity of the test<sup>9</sup>.

### Statistical analysis

The variables ML Flow test, sex, age, index case and BCG scar were analyzed and associations were evaluated using Pearson's

chi square test, considering a *p-value* < 0.25. Logistic regression for binary observations with 5% significance was used to verify the relation between predictable variables and the result of the ML Flow test. The *odds ratio* was estimated with a confidence interval of 95%.

## RESULTS

Of the 2,840 household contacts of leprosy patients submitted to the ML Flow test, 57.5% (1,632) were male, 73% (2,074) were household contacts of multibacillary cases of leprosy, 53.4% (1,517) had one BCG scar and 24.4% (692) two BCG scars. Most of the contacts (69.4%) were over 15 years-old. Patient age varied from 3 months and 18 days to 91 years-old, with a mean age of 27.7 years-old, a median of 15 years-old and standard deviation of 17.9 years.

Positivity in the ML Flow test among leprosy household contacts was 20.5% (582/2840). Positivity was higher among male household contacts (22.4%) than among females (17.9%), with a statistically significant difference (*p*=0.003) (**Table 1**). Positivity among household contacts of multibacillary cases was 23.9% (495/2074), while among paucibacillary cases, it was 10.8% (79/732) (*p*<0.001) (**Table 1**). The association between ML Flow test positivity and the number of BCG scars was not statistically significant (*p*=0.246) (**Table 1**). Positivity was 21.7% (427/1970) among household contacts aged over 15 years-old and 17.8% (154/867) for those 15 years-old or less (*p-value* = 0.017) (**Table 1**). The association between these variables and ML Flow test seropositivity in a descending order of chance were: household contact of a multibacillary case of leprosy (1.75), age over 15 years-old (1.38) and male household contact (1.25) (**Table 2**).

**TABLE 1**

Seroprevalence distribution of ML Flow in contacts, according sex, index case classification, BCG scar and age.

Variables	ML Flow Test				OR (CI 95%)	p-value*
	negative		positive			
	n <sup>o</sup>	%	n <sup>o</sup>	%		
Sex						
female	992	82.1	216	17.9	1.00	0.003
male	1,266	77.6	366	22.4	1.33 (1.10 – 1.60)	
Classification of Index case						
paucibacillary	653	89.2	79	10.8	1.00	<0.001
multibacillary	1,579	76.1	495	23.9	2.59 (2.01 – 3.34)	
BCG scar						
none	498	82.0	109	18.0	0.84 (0.64 – 1.1)	0.246
one	1,196	78.8	321	21.2	1.03 (0.83 – 1.29)	
two	549	79.3	143	20.7	1.00	
Age						
until 15 years old	713	82.2	154	17.8	1.00	0.017
more than 15 years old	1,543	78.3	427	21.7	1.28 (1.04 – 1.57)	

\*Pearson's Chi-square test, OR: odds ratio, CI: confidence intervals, BCG: bacille Calmette-Guérin.

**TABLE 2**

Multivariate analyses of seropositivity of ML Flow test in contacts of new leprosy cases (n<sup>o</sup>=2,840).

	OR	OR - CI 95%	
		low limit	high limit
Classification of index cases			
paucibacillary	1.00	—	—
multibacillary	1.75	1.33	2.31
Sex			
female	1.00	—	—
male	1.25	1.02	1.52
BCG scar			
none	0.72	0.53	0.97
one scar	0.96	0.76	1.21
two scars	1.00		
Age			
until 15 years old	1.00	—	—
more than 15 years old	1.38	1.10	1.72

Hosmer & Lemeshow (p - value=0.974). OR: odds ratio, CI: confidence intervals, BCG: bacille Calmette-Guérin.

**DISCUSSION**

ML Flow test positivity in leprosy household contacts is an indirect indicator of the dissemination of *Mycobacterium leprae* infection in the general population. This study showed 20.5% (582/2.840) seropositivity, with higher positivity among male household contacts, contacts of multibacillary cases and among those over 15 years of age. No correlation was observed between seropositivity and the number of BCG scars.

Seropositivity of 20.5% is in agreement with reports that showed ML Flow test seropositivity in leprosy household contacts of 28.6%<sup>9</sup> and of 15.6%<sup>10</sup>.

Seropositivity was higher (22.4%) among male contacts than females (p-value 0.003). No consensus exists in the literature regarding these data. Some reports showed higher positivity among females<sup>12 17 29 30 28</sup>, while others showed no difference between males and females<sup>10 23 2 28 4 26</sup>. Association with the leprosy index case showed that higher seropositivity occurred among household contacts of multibacillary cases of leprosy (23.9%, p-value <0.001). This finding is in agreement with the majority of the reports in the literature<sup>10 17 23 28 1 13 3</sup>. This fact is to be expected, since the probability of getting leprosy ranges from 5 to 14 times greater for contacts of multibacillary cases and twice as likely among contacts of paucibacillary cases<sup>27 32</sup>. Some reports observed no difference in seropositivity among household contacts of multibacillary and paucibacillary cases<sup>29 18 20 21 11</sup>.

Seropositivity was higher in contacts aged over 15 years-old. This is in agreement with data obtained by Calado<sup>10</sup>. Other authors have shown no differences<sup>28</sup> in seropositivity among age groups, or that it diminished<sup>30</sup> or increased<sup>29</sup> with age.

No difference was obtained in seropositivity among household contacts in relation to the number of BCG scars; a fact in agreement with the existing literature<sup>17 29 30 28 4</sup>.

It is important to stress that population surveys have suggested that subclinical infection is much more common than clinical

manifestation of the disease. Many risk factors influence the development of leprosy disease, such as contact with untreated multibacillary leprosy patients, with a high bacterial load and eliminating large amounts of bacilli in the environment. Other risk factors could also be involved, such as genetic traits, behavior, diet and hygiene habits, other concurrent infections or other predisposing factors in the house and or its surroundings<sup>17</sup>. Moreover, some studies have demonstrated that seropositive household contacts present a greater risk of developing leprosy in the future than seronegative household contacts<sup>12 29 13 15 14 24 7 8 4</sup>.

From an epidemiological view and considering public health actions, the goal of the correct treatment of index cases is an important part of disease control, but the value of contact surveillance must not be underestimated. This action must be supported and conducted systematically in order to examine and orient household contacts. This would enable more timely diagnosis and correct treatment and, in the near future, a decrease in the sources of infection in the general population and control of this endemic disease.

Although this study has shown that household contacts showed a greater chance of presenting a seropositive ML Flow test when in contact with a multibacillary leprosy patient, when aged over 15 years-old and when the contact was male, more studies are necessary to evaluate the role of seropositivity in contact surveillance. Follow-up of the seropositive and seronegative contacts during the four years after the application of the ML Flow test, may yield more information to help analyze the value of implementing this test in the leprosy control program.

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