Performance evaluation of an indirect immunofluorescence kit for the serological diagnosis of dengue

Avaliação do desempenho de kit de imunofluorescência indireta para o diagnóstico sorológico de dengue

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ABSTRACT

Objective: To evaluate the performance of indirect immunofluorescence for serological diagnosis of dengue virus in a population with high prevalence of arboviruses. Methods: Two-hundred serum samples from patients with clinical suspicion of dengue fever were tested by immunoenzymatic and indirect immunofluorescence assay BIOCHIP® mosaic. Specificity, sensitivity and Kappa coefficient were calculated. Discordant samples were tested by polymerase chain reaction for confirmation. Results: Of the 200 samples, 20% were positive and 80% negative for anti-dengue virus IgM antibodies in the immunoenzymatic test. Of the 40 positives, 25% were negative in indirect immunofluorescence. Of these ten discordant results, only 20% were also negative in the polymerase chain reaction (PCR). Of the 160 negatives in the immunoenzymatic test, 5% were positive in indirect immunofluorescence. Of these nine discordant results, 33% were positive in the PCR. The Kappa coefficient was 0.7 (0.572-0.829). Sensitivity and specificity of indirect immunofluorescence were respectively 75% and 94%. For anti-dengue virus IgG antibodies, of the 200 samples, 15.5% were positive and 84.5% were negative in the immunoenzymatic test. Of the 31 positives, 12.9% were negative in indirect immunofluorescence. Of these four discordant results, 25% were negative in the PCR. Of the 169 negatives, 8% were positive in indirect immunofluorescence. Of these 14 discordant results, 64% were also positive in the PCR. The Kappa coefficient was 0.695 (0.563-0.83). Sensitivity and specificity of indirect immunofluorescence were 87.1% and 91.7%, respectively. Conclusion: For diagnosis of acute infection, the immunoenzymatic test is enough, and the use of additional methods is not warranted. Replacing the immunoenzymatic test by indirect immunofluorescence would compromise the sensitivity for IgM. However, indirect immunofluorescence can distinguish three arboviruses simultaneously, an advantage during concomitant epidemics.

Keywords: Arbovirus infections; Dengue; Enzyme-linked immunosorbent assay; Serologic tests; Fluorescent antibody technique, indirect

RESUMO

Objetivo: Avaliar o desempenho da imunofluorescência indireta no diagnóstico sorológico de dengue em uma população com alta prevalência de arbovírus. Métodos: Duzentas amostras de soro de pacientes com suspeita clínica de dengue foram testadas por ensaio imunoenzimático e imunofluorescência indireta mosaic BIOCHIP®. Foram calculados especificidade, sensibilidade e coeficiente Kappa. Nas amostras discordantes, realizou-se reação em cadeia da polimerase como método confirmatório. Resultados: Das 200 amostras, 20% foram positivas e 80% negativas para IgM antívirus da dengue no ensaio imunoenzimático. Das 40 positivas, 25% foram
negative in the immunofluorescence indirect. Destas dey negatives, 
apenas 20% yam também negatives na reação em cadeia da 
polimerase. Das 160 negatives no ensayo imunoenzimático, 5% 
foram positivas na immunofluorescência indirecta. Por fim, dentre 
as nove discordantes, 33% tiveram virus da dengue detectado 
a reação em cadeia da polimerase. O coëfficiente Kappa foi 0,70 
(0,57-0,82). Sensibilidade e especificidade por immunofluorescência 
indireta foram, respectivamente, 75% e 94%. Para IgG antivírus da 
dengue, de 200 amostras, 15,5% foram positivas e 84,5% negatives 
novo ensayo imunoenzimático. Das 31 positives, 12,9% foram negatives 
a immunofluorescência indireta. Destas quatro discordantes, 25% 
apresentaram virus da dengue não detectado na reação em 
caedeia da polimerase. Das 169 negatives, 8% foram positives 
a immunofluorescência indirecta. Destas, 64% foram positives também 
na reação em cadeia da polimerase. O coëfficiente Kappa foi 0.695 (0,56- 
0,83). Sensibilidade e a especificidade por immunofluorescência 
indireta foram, respectivamente, 87,1% e 91,7%. Conclusão: Ensaio 
imunoenzimático seria suficiente para diagnóstico sorológico de 
infecção aguda, não justificando a incorporação da immunofluorescência 
indireta. Substituir ensayo imunoenzimático pela immunofluorescência 
indireta poderia comprometer a sensibilidade para IgM. Contudo, 
a immunofluorescência indireta auxilia diferenciar três arboviroses 
simultaneamente, sendo vantagoso em epidemias concomitantes. 

Descritores: Infecções por arbovírus; Dengue; Ensaio de imunoadsorção 
enzimática; Testes sorológicos; Técnica indireta de fluorescência 
para anticorpo

INTRODUCTION

Dengue fever, an arbovirus infection predominantly 
transmitted by vectors of the Aedes aegypti species, is 
a serious public health issue in Brazil, with seasonal 
epidemics virtually across the entire national territory.(1) 
The country recorded 572,308 probable cases in 2014; 
1,621,797 in 2015; 1,483,623 in 2016; 251,711 in 2017 
and 265,934 in 2018.(2-4) All four serotypes of the dengue 
virus are present in Brazil.

Laboratory screening for the dengue virus mostly 
involves techniques of viral isolation, identification of 
dengue virus (DENV)-specific antibodies using serologic 
tests, direct identification of viral RNA, and detection 
of the NS1 antigen.(5-7) 

The serologic diagnosis of acute infection is based 
on detection of DENV-specific immunoglobulin M 
(IgM), detectable in 93% of cases, 6 to 10 days after the 
onset of fever.(6) Dengue virus-specific immunoglobulin 
G (IgG) can be detected in current infections if, at the 
time the test is performed, seroconversion has already 
taken place, and is otherwise useful to check for past 
infections. The IgG avidity test helps differentiate 
between primary and secondary infections by the 
dengue virus.(6) 

The Enzyme-Linked Immunosorbert Assay (ELISA) 
is currently the most commonly used serologic test in 
clinical laboratories. It is a simple, quick test requiring 
limited high-tech equipment.(6-11) During an epidemic, 
the ELISA assay can quickly determine the extent of 
transmission. In dengue-endemic areas, this test can be 
used to screen a large number of serum samples at low 
costs.(11)

Indirect immunofluorescence is another sorologic 
method to identify dengue virus-specific IgM and IgG 
antibodies, however few studies(12,13) have investigated 
the use of this test. Most serologic tests available in 
clinical laboratories in Brazil were developed 
abroad, and their validation studies were frequently 
conducted in populations in which the disease is not 
highly prevalent and which have not experienced 
concomitant outbreaks of other arboviruses, such as 
Zika and Chikungunya, and this could lead to false-
positive results for dengue virus due to cross-reactive 
antibodies, hindering diagnosis.(14)

During the dengue fever epidemics in 2016, a new 
indirect immunofluorescence (IIF) test, the BIOCHIP® 
mosaic developed in Germany and promising to 
serologically detect the dengue, Zika and Chikungunya 
viruses, was released in Brazil to compete with ELISA 
assays, which had been used as routine for a longer time.

OBJECTIVE

To evaluate the diagnostic performance of an indirect 
immunofluorescence assay for serologic diagnosis of 
the dengue virus in a population with high prevalence 
of arboviruses, in comparison with the ELISA sorologic 
test.

METHODS

We used 200 serum samples from routine testing at the 
Clinical Laboratory of Hospital Israelita Albert Einstein 
(HIAE), collected in 2014 and sent to the laboratory 
due to clinical suspicion of infection by the dengue virus. 
Samples were characterized at the time as negative or 
positive for dengue virus using ELISA (Focus, USA). 
All samples were tested with the BIOCHIP® mosaic IIF 
technique (Euroimmun, Germany).

The appropriate statistical tools (EP Evaluatoar 
software) were used to calculate pre-test probabilities: 
sensitivity (diagnostic test’s ability to detect true positive) 
and specificity (diagnostic test’s ability to detect true 
negative). We also calculated post-test probabilities: 
positive predictive value (rate of patients with positive 
tests who effectively have the disease according to the 
gold standard test), negative predictive value (rate of 
patients with negative tests who effectively do not have 
the disease according to the gold standard test). Finally,
we calculated accuracy, which is the probability of the test providing correct results, and the Kappa coefficient, a measure of the level of agreement between two methods, adjusted by the odds, i.e., it informs the non-random chance, ranging from -1 to 1, where 0.00 is no agreement, 0.00-0.20 is poor agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 good agreement, 0.81-0.99 very good agreement, and 1 is perfect agreement.\(^{(15,16)}\)

In samples for which the two methods were discordant, we used polymerase chain reaction (PCR) as a confirmatory diagnostic method. Polymerase chain reaction is a molecular assay which quantitatively detects DENV RNA, and is considered the gold standard, since it can effectively prove that the virus is present in the body. However, it has limitations, including its high cost, which prevents it from being routinely used for screening in laboratories.\(^{(5)}\)

This study was approved by the Institutional Review Board of Hospital Israelita Albert Einstein, CAAE: 83521718.5.0000.0071, opinion nº 2.909.625.

## RESULTS

Of the 200 samples studied, 40 (20%) were classified as positive and 160 (80%) as negative for anti-DENV IgM antibodies, in the reference ELISA test.

Of the 40 positive samples, 10 (25%) were negative in the IIF test; of these 10 discordant samples, only 2 (20%) were also negative in the PCR. However, of the 160 negative samples in the reference ELISA, 9 (5%) were positive in the IIF test; of these 9 discordant samples, 3 (33%) were positive for DENV in the PCR (Table 1).

Table 1. Detection of anti-dengue virus IgM antibodies in the ELISA and indirect immunofluorescence tests

| IgM indirect immunofluorescence | IgM ELISA | Total |
|--------------------------------|-----------|-------|
| Positive                       | 30        | 39    |
| Negative                       | 10        | 161   |
| Total                          | 40        | 160   |

When the Kappa test was used on results from both assays, an agreement level of 0.695 (0.563-0.83) was found. The sensitivity and specificity of indirect immunofluorescence was 87.1% and 91.7%, respectively. The positive predictive value of IIF was 65.8%, the negative predictive value was 97.4%, and accuracy, 91%.

## DISCUSSION

The level of agreement, as verified by the Kappa coefficient, between the new IIF test and the test used as reference ELISA was acceptable, attesting to the good performance of the new test.

However, PCR, which was the method used to confirm the presence of viral antigens in samples for which the two methods were discordant, showed higher agreement with ELISA in most cases, except those with negative ELISA and positive IIF for anti-DENV IgG antibodies (false-positive IgG), where the PCR was 64% concordant with indirect immunofluorescence.

## CONCLUSION

Indirect immunofluorescence has acceptable performance, however, for clinically relevant situations, when diagnosing acute infection (detection of IgM antibodies), ELISA alone is sufficient for serologic diagnosis, and the use
of an additional method is not warranted. Replacing ELISA with indirect immunofluorescence, in turn, could compromise diagnostic sensitivity, increasing the number of false-negative samples for IgM.

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