

# Assessment of Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of *Mycoplasma Pneumoniae* Infection in Paediatric University Hospital

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## Abstract

**Introduction:** Community-acquired pneumonia (CAP) caused by *M. pneumoniae* affect 3%-10% of children. It is not possible to spot *M. pneumoniae* infection depending entirely on clinical signs and symptoms. Correct identification of *M. pneumoniae* infections is crucial to initiate proper antibiotic therapy. The role of culture is questionable as this organism is fastidious. Diagnostic accuracy of serology depends on the specimen collection time. IgM can usually be tracked within one week after the onset of clinical illness, followed by IgG two weeks later. Nucleic Acid Amplification Techniques (NAATs) are proved to be the “new gold standard”. Loop-Mediated Isothermal Amplification (LAMP) amplifies DNA of *mycoplasma* in less than an hour under isothermal conditions with the use of six primers in a single tube. The amplified products can be visualized by naked eye as turbidity or fluorescence.

**Aim:** To assess the diagnostic value of LAMP assay compared to serum *Mycoplasma* IgM for rapid detection of *M. pneumoniae*.

**Materials and methods:** A 6-months study was conducted on hospitalized children diagnosed with community acquired pneumonia (CAP) admitted to Cairo University specialized pediatric hospital (CUSPH). *M. pneumoniae* IgM was done on serum samples by RD-Ratio Diagnostics ELISA kit, Germany. LAMP assay was done on nasopharyngeal swabs using Loopamp™ RNA/DNA, Eiken Chemical, Japan. Kappa measure of agreement was calculated to assess the concordance between LAMP and *Mycoplasma* IgM.

**Results:** 90 hospitalized children with CAP were included in the study. Serum IgM was positive in 27(30%) of cases. LAMP assay was positive in 33 cases (36.7%). The sensitivity of LAMP was 85.2%, the specificity was 84.1%, PPV was 69.7%, NPV 93.0% with AUC=0.847. The agreement between serology and LAMP was good K=0.652 with 95% CI (0.487-0.816).

**Conclusion:** LAMP technique is a rapid, sensitive and specific method for diagnosis of *M. pneumoniae*. Combination of LAMP assay and serology may be optimal for the diagnosis of *M. pneumoniae* infection.

## Keywords

Pneumonia • Serology • LAMP technique • Diagnosis

## Introduction

The incidence of *M. pneumoniae* (MP) Community-Acquired Pneumonia (CAP) in children is estimated to affect 3-10% of children, and less than 5% of CAP cases are severe enough to require hospitalization [1]. Correct identification of *M. pneumoniae* infections is vital for prescription of the appropriate therapy since, based on clinical signs and symptoms, detection of *M. pneumoniae* infection is not possible [2]. Because MP is a fastidious organism, the role of culture is questionable and nearly abandoned the practice of laboratory diagnosis of this infection, in favor of serology and molecular detection [3].

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**Received** January 27, 2021; **Accepted** February 10, 2021; **Published** February 17, 2021

Diagnostic accuracy of serology of acute *M. pneumoniae* infections depends on the specimen collection time and performance characteristics of the used test. IgM can usually be tracked within one week after the onset of clinical illness, followed by IgG two weeks later. A gradual tapering occurs over months to years [1].

The higher sensitivity and shorter turnaround time of Nucleic Acid Amplification Techniques (NAATs) led to its consideration as the “new gold standard” [4].

Loop-Mediated Isothermal Amplification (LAMP) was [5] developed by Notomi et al. This technique depends on the rapid amplification of *Mycoplasma* DNA, in less than an hour under isothermal conditions using a set of four to six primers, measuring eight sequences of a target gene with all the reagents incubated in a single tube. The amplification products can be seen by visual detection of turbidity or fluorescence. This method is considered an attractive mode of surveillance of infectious diseases in developing countries because it does not need sophisticated equipment or skilled personnel requirement [6].

In the present study, we aimed to assess the diagnostic value of LAMP assay compared to serum *Mycoplasma* IgM for rapid detection of *M. pneumoniae*.

## Methodology

A 6-months study was conducted on hospitalized children diagnosed clinically as Community Acquired Pneumonia (CAP) admitted to the pediatric ward or pediatric ICU of Cairo University Specialized Pediatric

Hospital (CUSPH). Ethical committee of Cairo University Specialized Pediatric Hospital (CUSPH) approved the study. Informed consent from the parents or guardians was obtained for participating in the study. Samples were taken within the first 2 days of admission to the hospital.

### M. Pneumonia serology

*M. pneumoniae* IgM was done by RD-Ratio Diagnostics ELISA kit, Germany, Catalog No. E-MCG-K42 on serum samples according to the manufacturer instructions.

Loop mediated isothermal Amplification (LAMP) assay: using LMP 247 Loopamp™ RNA/DNA Amplification Reagent D, Eiken Chemical, Japan.

**Nasopharyngeal sampling:** Nasopharyngeal swab was used for sample collection from the nasopharynx. Once the sample is collected the swab was placed immediately into the Copan Universal Transport Medium (UTM-RT) catalog number 305°C which can maintain even fragile organisms for long periods of time at room temperature.

**DNA extraction:** The samples were concentrated from 2 ml to 200 µl according to the manufacturer protocol for sample concentration and the product then underwent DNA extraction following the manufacture instructions of QIAamp® DNA Mini kit catalog no. #51306.

**LAMP primers:** Five LAMP primers (Bio search technologies) (Table 1) for detection of P1 adhesion gene of *M. pneumoniae* as [7] described before by Saito et al.

Primer name	Sequence	No. of bases	Total n mol
F3: Forward Outer Primer	5' CACCCCTCGGGGGCAGTCA 3'	18	45.13
B3: Backward Outer Primer	5' CTGATTGTTCCCTGCTGGT 3'	18	52.32
Forward Inner Primer (FIP)	5' GTTCAGAGCTGGAGGTTGGC CGATGATTACAGGCGGTTTC 3'	39	45.06
Backward Inner Primer (BIP)	5' GGGCGGGGTGAAGGAATGA CTCGTGAAGTTGGTGTGG 3'	37	36.86
Loop Forward Primer (LFP)	5' AGGCTTCAAGTGGACAAA GTGAC 3'	23	45.88

Table 1. LAMP primers.

The reaction tubes were placed into thermal cycler (SEE AMP™, Seegene) to start the reaction at 65°C for 60 minutes followed by enzyme inactivation at 80°C for 5 minutes.

### Fluorescent/visual detection

The samples were examined on UV lamp spectroline® with a wave length 365 nm. The reaction tubes were observed from the sides of the tubes with eyes protected by eyeglasses (Figure 1).

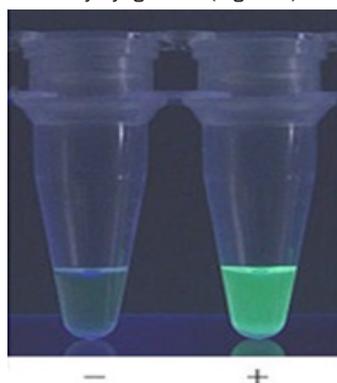


Figure 1. Results of LAMP by fluorescence.

**Positive samples:** Produce green fluorescence.

**Negative samples:** Produce no fluorescence.

*Mycoplasma pneumoniae* M129 (strain: ATCC 29342) was used as positive control and DNase/RNase-free sterile water as negative control. The total measurement time of this assay was approximately 2 hours.

**Statistical analysis:** Data was entered and analyzed through the Statistical Package of Social Science Software program, version 23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

## Results

The present study included 90 hospitalized children with community-acquired pneumonia. The age of the studied cases was from one month to eight years with mean and SD 8.3+13.2 months and median with IQR 4(2-10) years.

Forty-two cases (46.7%) were studied in winter season and 48(53.3%) in early summer. Serum IgM for *M. pneumoniae* was positive in 27(30%) of cases. LAMP assay for detection of *Mycoplasma* was positive in 33 cases (36.7%). We used IgM serology as our gold standard for diagnosing MP [4]. The sensitivity of LAMP was 85.2%, the specificity was 84.1%, PPV was 69.7%, NPV 93.0% with AUC=0.847. The agreement between serology and LAMP was good K=0.652 with 95% CI (0.487-0.816).

## Discussion

The present study aimed to evaluate the LAMP assay as a novel nucleic acid amplification method, for the detection of *M. pneumoniae* in nasopharyngeal specimens.

It is a common belief that MP incidence is higher in school-aged children and young adults; hence the name of "walking pneumonia" is coined for this infection. Few studies, however have reported the frequency of *M. pneumoniae* infections in infants [8].

All of our MP positive cases were below 5 years of age with median of 4 years. In contrast most countries in worldwide with the incidence being much higher in children more than 5 years [9-12].

30% was the incidence of MP CAP in our hospitalized children; which is higher than other reported studies. Incidence of MP is ranging (6%-18%) among hospitalized children worldwide [1,11,13].

With the results of IgM serology being considered as standard, LAMP assay had 85.2% sensitivity and 84.1% specificity with good agreement with the serological results K=0.652(0.487-0.816) [4].

Other studies reported higher sensitivity and specificity of LAMP 94.8%, 91.9% respectively in pediatric patients with MP pneumonia [4].

The relatively lower sensitivity and specificity of LAMP assay in our study may be due to the fact that the fluorescence kit is too sensitive for humidity and once it is opened the total number of the tests should be consumed otherwise false positive fluorescence is observed. Moreover, judgment of fluorescence is sometimes difficult so studies that evaluated turbidimetric LAMP reported more favorable results with 100% and the specificity [14].

In the present study, 27(30%) positive cases for IgM, 4(4.4%) were tested LAMP negative. These false negative LAMP results may have been caused by the disappearance of potential causative organisms resulting from treatment with previous antibiotics as all of the patients have been treated with antibiotics in the first day of their infection.

Similarly, Kakuya et al. have observed positive results of IgM in patients with negative LAMP assay and stated that this may be due to the fact that *M. pneumoniae* IgM antibodies may persist for several weeks to months as

those patients had no current but a relatively recent infection [15].

In accordance with our results, Morozumi et al. reported that PCR positivity was significantly lower among patients who were infected with *M. pneumoniae* and received antibiotics than among those who did not receive antibiotics [16].

On the other hand, 10 cases were positive by LAMP assay and had negative IgM. Several reports have demonstrated a weak or deferred antibody response to *M. pneumoniae* in young children or patients in the early stages of disease [17]. The main cause of false negative results of single IgM measurement by EIA is the short time interval between the onset of illness and sample collection.

Kakuya et al. Stated that it is possible that reinfection with *M. pneumoniae* even in children may be accompanied by the production of undetectable levels of IgM that could be another cause for false negative results for single measurement on serological testing [15].

Furthermore, Daxboeck et al. stated that transient asymptomatic carriage of *M. pneumoniae* may result from the persistence of the pathogen after previous disease and from infections during incubation periods. Asymptomatic healthy children act as carriers of *M. pneumoniae* at a rate of 0%-2.2% [18,19]. Consequently; the detection of *M. pneumoniae* DNA in throat swabs is not necessarily indicative of a causative role of the pneumonic pathogen, an infectious state is often difficult to distinguish from carriage during the acute phase [4].

So, Gotoh et al. concluded that a combination of the LAMP assay and serological tests may accordingly be optimal for the diagnosis of *M. pneumoniae* pneumonia [4].

LAMP does not require a thermal cyclor and can be performed simply with a heating block and/or water bath. The advantages of rapid amplification, simple operation and easy detection give LAMP the opportunity for applications in clinical diagnosis as well as surveillance of infectious diseases in developing countries without need of sophisticated equipment or skilled personnel [20].

## Conclusion and Recommendations

*Mycoplasma pneumoniae* is an important causative agent of CAP in Egyptian children. Diagnosing MP is challenging, as the methods of detection of this organism have some pitfalls. The serologic methods used, the time lapse needed to augment an immune response to form IgM antibodies, following recent infection, the necessity for acquiring a paired serum sample two weeks after the onset of symptoms for antibody testing, which may be inaccessible in clinical practice, and the cross-reactions with other pathogens, can all affect the results interpretation. A rapid diagnostic method is essential in prescribing the effective antibiotics. LAMP is a novel gene amplification technique, helps in simple quick diagnosis and early detection of microorganisms.

According to our study LAMP is concluded as a rapid, sensitive and specific method for diagnosis of *M. pneumoniae*. Combination of LAMP assay and serological tests may be optimal for the diagnosis of *M. pneumoniae* infection.

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**How to cite this article:** Rahman, Eiman M Abdul, Amal Mohammed Sayed, Reham H.A. Yousef and Bassant Salah Saad et al. "Assessment of Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of *Mycoplasma Pneumoniae* Infection in Paediatric University Hospital." *Med Microb Diagn* 10 (2021): 314.