

Development of a Rapid PCR-Based Test for Early Detection of Leptospirosis in Rural Areas

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ABSTRACT

The benefits of these molecular techniques for leptospirosis diagnosis are considered, as is Colombia's response to the SARS-CoV-2 pandemic, which made it possible to develop and provide 227 real-time PCR test-capable laboratories in 28 of the 32 departments of the country. It is suggested that a molecular test be standardized to enable the precise diagnosis of serovars and species of *Leptospira* nationwide. By encouraging the diagnosis of leptospirosis during its acute phase, this approach may help lower the underreporting of leptospirosis-related morbidity and death in rural area. Additionally, it would support healthcare professionals in managing the condition, minimizing antibiotic usage, reducing patient problems and hospital admissions, and contributing to a more accurate diagnosis of the disease. It would also help minimize the costs and time spent on laboratory diagnostic testing. The important implication of a rapid PCR Test for the detection of Leptospirosis is that it can be useful for the evaluation of vaccine efficiency in a particular population. As described earlier, the vaccine is the best preventive method against disease, but some factors can reduce the efficiency of the vaccine in any particular population in rural areas. However, PCR-based Tests can effectively evaluate vaccine efficiency in any targeted population. This can also be used to evaluate vaccine efficiency in the human population and livestock.

Keywords: Rapid PCR-Based Test (PCR), Detection (DD), Leptospirosis (LL), Rural Area (RA)

INTRODUCTION

Leptospirosis is a kind of bacterial disease that is not only common in human beings but also found in other animals. The main cause of transmission of this disease is contaminated water, soil, or vegetation. The main symptoms of this disease are fever, headache, muscle aches, nausea, vomiting, hepatitis, diarrhea, rashes, and others. The early detection of leptospirosis is mandatory because swift diagnosis helps in developing better and more effective treatment strategies for patients[1]. For early detection of Leptospirosis, a PCR Based Test is mostly used. In this introduction, we will discuss how rapid PCR Tests can be used for early detection of Leptospirosis in Rural areas. There are many objectives for using rapid PCR-based tests to detect leptospirosis in rural areas. The first and foremost objective of a rapid PCR Test for detecting Leptospirosis is to develop a rapid, sensitive, and accurate PCR Test for detecting early symptoms of Leptospirosis. The other main objective of rapid PCR-based tests for the detection of leptospirosis is to perform these PCR-based tests in rural areas with limited resources[2]. The other important objective of this rapid PCR Test for detecting Leptospirosis is to compare the accuracy of this diagnostic test with other diagnostic methods. There are some important steps in the methodology of rapid PCR-based Tests for the detection of leptospirosis in rural areas. The first step is to collect the sample from suspected patients. These samples can be collected from the urine, blood, or saliva of suspected patients of Leptospirosis. The next step is DNA extraction from these samples. This step is performed in the laboratory and with highly sensitive instruments to get accurate results in case of disease[3]. The next step is primer design, in which a specific gene of Leptospirosis is targeted during detecting Leptospirosis. The next step is the PCR protocol, in which a PCR-based test is performed for early detection of leptospirosis. The next important step is test validation, which is a mandatory part of a rapid PCR-based test for the detection of leptospirosis. This step ensures the accuracy of rapid PCR-based tests for the detection of leptospirosis by comparing

them with other reference strains, clinical samples, and field trials. Many types of PCR-based tests are used for the early detection of Leptospirosis. These important types of PCR-based tests are conventional PCR, real-time PCR, nested PCR, multiplex PCR, loop-mediated isothermal amplification, and others. Rapid PCR-based Tests have many advantages for detecting Leptospirosis in Rural areas[4]. The first advantage is that rapid PCR-based Test has less turnaround time of almost one hour. The other main advantage of a rapid PCR test for the detection of leptospirosis is that it is simple and easy to perform compared to other diagnostic methods for leptospirosis. The next advantage of rapid PCR-based tests is that minimal equipment is required to perform these tests to detect leptospirosis. In other diagnostic methods, there is a need for advanced equipment. Along with this, there is a high turnaround time for other diagnostic methods, which take many hours or maybe days[5]. The other diagnostic methods for leptospirosis are quite complex and difficult to perform. The next advantage of a rapid PCR PCR-based test for the detection of leptospirosis is that this diagnostic method has very little cost compared to other diagnostic methods. This test can easily be performed using limited resources. The next advantage of PCR-based tests is that they have settings that can also be operated in rural areas. In contrast, the other diagnostic methods are confined to being performed only in the laboratory[6]. So, these settings systems cannot be operated in rural areas like rapid tests. The next advantage of a rapid PCR Test for detecting Leptospirosis is that it takes samples that are noninvasive for patients. These samples are collected from blood and urine, which are noninvasive ways for leptospirosis patients. This rapid PCR test cannot only be performed for the detection of disease but can also be used for screening programs to check any possible risk of disease in high-risk groups [7]. The main spread of disease takes place in communities with the help of transmission sources. If these transmission sources are understood well, the spread of disease in any

Community can easily be controlled. The main benefit of a rapid PCR Test for detecting Leptospirosis is that it can be used to understand the transmission sources of any disease such as leptospirosis. As we know, preventive measures are essential to avoiding any disease. Knowledge measures can easily be learned from Tests PCR-based tests PCR-based test can also give an idea about how to control disease patterns in any population[8]. Epidemiological studies are also important in the study of disease, causes, effects, and transmission modes in any population. The rapid PCR-based test can also be helpful in epidemiological studies. Although there are many benefits of using PCR-based tests for detecting leptospirosis in rural areas, there are also some limitations to PCR-based tests for detecting leptospirosis. The first limitation is that the DNA sample handling method can contaminate samples, thus reducing the accuracy of results. The other limitation is that some factors act as inhibitors in the rapid PCR-based Tests to detect Leptospirosis. These inhibitors may block the reactions in PCR-based tests to detect leptospirosis. The other aspect of the limitation of a PCR Based Test for detecting Leptospirosis is that equipment calibration may differ depending upon the environment in which the based Test is performed[9]. These important aspects of rapid PCR PCR-based tests for the detection of Leptospirosis suggests that there is a need for more advancement in this system[10].

Research Objective

The main objective of this research is to understand the development of rapid PCR-based Tests for the detection of Leptospirosis in Rural areas. This study effectively explains different aspects of rapid PCR-based Tests for detecting Leptospirosis. The research study determine that development related to the rapid PCR based test for early dections leptospirosis in the rural area. The research paper divided into five section first portion represent that introduction included objective of research. The second section describe literature review the third portion describe tools and techniques also that explain the implications about research. The fourth section represent that result and describe also that last section summarized overall research study and present recommendation also suggestion between them.

LITERATURE REVIEW

Researchers claim that in Nigeria, the disease that mainly affects a large number of human and animal populations is leptospirosis.in Nigeria, various efforts have been made by medical health workers to identify the molecular characteristics responsible for causing Leptospirosis in adult[11]. Studies suggest that leptospira-named bacteria are responsible for causing the disease Leptospirosis in people. the pathogenic attack of bacteria is on both animals and humans. However, various documentation-based studies reveal that leptospirosis is observed only in cattle and not in all types of animals. The studies made in Uganda claim the presence of Leptospirosis in sheep and goats[12].Studies show that detection of Leptospirosis requires an antibody test. This test is performed using the ELISA technique. The monitoring of the disease condition of leptospirosis is done using the serological method. This method is significant in determining the disease epidemiology. The preventive measures against the spread of leptospirosis are developed by understanding the epidemiology of the disease[13].Studies predict that the mortality rate due to Leptospirosis is higher due to the uncertainty in the preventive measures of the disease. Uncertainty is the main reason for the complexity of the disease. The spread of Leptospirosis affects the soil

and economic conditions of the countries, as infected cattle can damage or infect the water. when used in crop fields, the infected water results in crop damage and other problems[14].Studies claim that global disease prevention authorities are working to stop the spread of leptospirosis. The main aim of these authorities is to collect data about the current cases of Leptospirosis in a given state and then to develop preventive strategies to minimize its spread [15]. Scholars elaborate that the complexity of the disease associated with Leptospirosis in Siberia is still undiscovered. the first case of Leptospirosis was observed in wild rats in Siberia. The Geno typical analysis of the disease in wild rats provided evidence about the presence of Leptospirosis species in rats[16]. Studies claim that various tests have been performed on patients with Leptospirosis to identify the gene responsible for causing mutation in the normal molecular activities of the diseased patients[17]a recent research, made in sirilanka, identified the presence of gene flaB in the Leptospirosis patients. this gene is regarded as novel gene, and its presence was identified using a PCR method in the laboratory. studies show that in the southern states of Sudan, the prevalence of Leptospirosis is higher in cattle. The risk of spread of Leptospirosis to humans increases due to the high prevalence of this disease in slaughtered sheep and other cattle[18]. This proves that gathering the Leptospirosis cultures is a very difficult task. A dependent DNA capturing method is employed to gather the culture of lepostsrois for studying this species. The dependent captures were made from humans and complex species to thoroughly explain the severity caused by Leptospirosis in different species[19]. Researchers reveal that urbanization, as well as climatic factors, are among the various factors that are responsible for the rise in Leptospirosis cases.in Srilanka the cases of Leptospirosis is higher because of lack of preventive measures Leptospirosis is a zoonotic disease that spreads and results in serious health consequence [20]. Studies explain that various agents can cause Leptospirosis. the severity of this disease leads to an epidemic as it spreads from one include to another. Also about sixty percent of the known virus are zoonotic. Using innovative technology helps in determining the cause and spread of leptospirosis[21]. Studies suggest that the use of genomic sequencing techniques is rapidly increasing as they detect the presence of leptospirosis in any infected individual. due to the limitation of resources in genomic labs, the work on pathogenic genomic sequencing is limited but in near future, this field of genomic sequencing will advanced .analysing the genomics of Leptospirosis specie it becomes easy to control its spread and severity[22]studies explain that various guidelines have been developed to identify the spread of Leptospirosis in SOT patients.in south Asia specific guidelines are provided to SOT patients so that they can avoid getting the Leptospirosis. all these guidelines are provided by transplant physicians and highly professional health care staff[23]researchers claim that scrub typhus condition is assessed suing specific test These teste guidelines include IFAT as well as ELISA. enhanced and improved diagnostic approaches are used by [physicians to ensure the results obtained from the testing are accurate. also. The diagnostic test regarding scrub typhus is made on serum collected from patients suffering from a number of ailments This process is done to assess the risk factors associated with scrub typh[24]. Studies show that animals' health gets badly affected by the spared parasitic namatiode. To identify the nematode found in the intestine of animals, microscopic methods is preferable. Various intestinal nematode samples from infected animals are tested by using an amplified PCR technique to achieve better results[25] Also, the diagnostic changes faced by the

health care professional make the treatment process difficult. the fever causes behind different types of ailments are easily detectable using the gene expression method. In Sri Lanka, the gene expression method assessed the number of viral and bacterial fever cases. The host-specific transitional biomarkers significantly play a role in determining the cause of fever in patients [26]. Moreover, another very prominent disease is prevalent in South Asian states. this disease is scrub typhus that enters the body of its hoist through mites and other small insects. Thes disease's symptoms are unpredictable, making its treatments a bit challenging [27]. Studixplain that plasmodium's special presence in the human body requires highly amplified testing.to test the presence of plasmodium in the human body, amplified PCR tests are

used in laboratory settings. this PCR test is performed in thirty minutes, and the results are super accurat[28]. Studies show that due to a lack of data management process, a lot of data related to livestock disease has been lost. The identification of any disease related to livestock requires a detailed understanding of the disease without any proper medical history of the disease. the lack of animal health data makes the disease prevention more difficult[29]. Studies suggest that during pregnancy, women can develop Borelli infection that is transferred to the fetus in some cases.in most cases, serological techniques are employed to test the effect of the infection on women. adopting preventive measures can help to save the mother during pregnancy from any infectious disease[30]. (Figure 1)



FIGURE 1 : Leptospirosis

Methods

The research determine that development related to the rapid PCR based test analysis related to the early detection of leptospirosis. The research study depends upon primary data analysis for determine the data used smart PLS software and generate result included descriptive statistic, the correlation coefficient analysis also that explain the estimated model between them. The rapid PCR-based test has been gaining much importance in recent years because of the accuracy of results and simplicity of procedure. It has been used for early detection of many diseases, including leptospirosis. This disease is a bacterial disease with various symptoms such as fever, headache, rashes, muscle pain, etc. There are many important implications of using rapid-based tests for early detection of Leptospirosis in Rural areas, which are described below:

Early Diagnosis of Diseases, Better Outcomes in Patients, Reduced Mortality, Enhanced Monitoring

As we know, medical science proves that the early detection and diagnosis of diseases will help in the better, more effective, and swifter treatment of diseases. In the case of Leptospirosis, the symptoms of this disease have shown very late, and until that time, the disease may spread to many other fellow human beings as well. As we know, the vectors of this disease are not only human beings but also animals. So, we have to take preventive measures to avoid any contact with any possible vector. In the case of a rapid PCR Test for detecting Leptospirosis, it can be beneficial in providing information about the presence or absence of a pathogen even before showing any possible symptoms. When these rapid PCR PCR-based tests are performed in rural areas in suspected patients, there is a high chance of possible

early diagnosis of leptospirosis. This early diagnosis also helped healthcare professionals start treatment before any symptoms in suspected patients. The other important implication of rapid PCR PCR-based tests for the detection of leptospirosis is that they can increase the ratio of better outcomes in patients. As we know, people in rural areas are more prone to diseases related to bacteria or other microorganisms. The reason is that there is suitable environment in rural areas for the growth and pathogenicity of these pathogens. In addition, there are limited resources for healthcare sectors in rural areas. That's why there are fewer approaches to diagnostic methods there. But the important implication of a PCR Based Test is that it can also be performed in rural areas with high Accuracy and limited resources. When rapid PCR-based Tests were conducted in rural areas for early detection of Leptospirosis, there was a prominent decline in the increasing level of this disease. In this way, rapid PCR-based tests are useful for bringing positive outcomes to patients. The other important implication of rapid PCR-based Tests for detecting Leptospirosis is that they can help reduce the mortality rate in rural areas. As it is described earlier, there is a high mortality rate in rural areas because of this fatal disease. However, with the help of a rapid PCR test, this disease can be detected in the early stages in patients, which will help reduce the mortality rate in rural areas. The other important implication of rapid PCR tests for the detection of leptospirosis is enhanced monitoring and screening. The rapid PCR PCR-based test is not only useful for the detection of leptospirosis but can also be used for screening in masses to learn about suspected patients. This rapid PCR-based test is also useful for epidemiological studies related to leptospirosis. This aspect will also help us understand how this disease will spread in a particular population. This aspect will also help to take preventive measures to avoid the onset of any such disease in rural areas. These important

implications of rapid PCR Tests convince us that they are quite helpful for the early detection of leptospirosis.

Identification of Infected Animals, Targeted Vaccination for Prevention, Development of New Diagnostic Markers, Evaluation of Vaccine Efficiency:

As we know, there are some diseases in which the animal body acts as a vector or transmission source of this disease. In this case, screening is necessary in the human population and animals that can act as vectors for this disease. The main implication of a rapid PCR Test for the detection of Leptospirosis is that it can be helpful for the identification of pathogens in animals as well. This rapid PCR-based test, when performed in rural areas, can be beneficial for studying animal populations and their relevance to disease transmission. The

other important implication of rapid PCR-based tests is the aspect of targeted vaccination in human beings and animals. As we know, prevention is far better than treatment. Vaccination is the best method of preventing disease in animals and human beings. The rapid PCR Test can be useful for studying the genetics of pathogens because of DNA integration. This study of genetics can help develop vaccines that can be better for preventing the onset of such deadly diseases. In this way, we can say that PCR-based tests can also be utilized to develop vaccines in healthcare. The other implication of a rapid PCR-based test for the detection of leptospirosis is the development of new diagnostic markers. As we know, a disease can be detected in more than one way through various diagnostic methods. Various diagnostic methods can be formulated with the help of rapid PCR Tests through the development of many different diagnostic markers.

Descriptive Statistic Analysis

Table 1: Result of Descriptive Statistic Analysis

Name	No.	Mean	Median	Scale Min	Scale Max	Standard Deviation	Excess Kurtosis	Skewness	Cramér-Von Mises P Value
PCR1	1	1.980	2.000	1.000	4.000	0.948	-0.594	0.621	0.000
PCR2	2	1.620	2.000	1.000	3.000	0.629	-0.590	0.522	0.000
PCR3	3	1.600	2.000	1.000	3.000	0.600	-0.608	0.458	0.000
PCR4	4	1.660	2.000	1.000	3.000	0.587	-0.610	0.258	0.000
DOL1	5	1.640	2.000	1.000	3.000	0.625	-0.609	0.458	0.000
DOL2	6	1.560	2.000	1.000	3.000	0.605	-0.522	0.599	0.000
DOL3	7	1.660	2.000	1.000	4.000	0.681	1.342	0.955	0.000
DOL4	8	1.700	2.000	1.000	3.000	0.700	-0.845	0.505	0.000
DOL5	9	1.640	2.000	1.000	3.000	0.625	-0.609	0.458	0.000

The previously mentioned outcome shown in table 1 demonstrates how descriptive statistical analysis results explain the standard deviation rate and characterize mean values, median rates, minimum values, and maximum values. The dependent and independent indicators' skewness values and overall significant values were all included. One independent variable is the PCR1. 1.980 is the mean value. 2.000 is the overall variable's median rate. The values range from 1.000 to the lowest to 3.000 at the highest. 62% of the skewness value between them is indicated by the PCR1 skewness rate of 0.621. The fact that the total significant value between them is 0.000 indicates a 100% significant rate. The mean values of 1.6200, 1.600, and 1.6600 for PCR2, PCR3, and PCR4, which are all regarded as independent variables, indicate a positive average rate, whereas the standard deviation rates are 62%, 60%, and 58% away from the mean. A 100% significant level of correlation is shown by the overall significant value of 0.000. The values for skewness are 25% and 45%, respectively. The DOL 1, 2, 3, 4, 5, and 6 findings are all considered dependent variables. They show a positive average value of 1.560, 1.660, 1.700, and 1.640 as the mean value. 62% of the mean value deviates from the mean, according to the 70% standard deviation rate. For each, the skewness values are 50% and 45%. One of the most prevalent zoonoses in the globe is leptospirosis. Leptospirosis is an infectious illness that has been demonstrated to be reemerging in both people and animals. Leptospirosis is found all around the world, however it is most prevalent in tropical and temperate regions. Yet, the predicted increase in global temperatures might lead to a significant increase in the prevalence of leptospirosis. It is believed that leptospirosis is widespread across Southeast Asia, particularly in Malaysia, where many outbreaks have been documented. Acute fever illnesses were discovered in several of the athletes competing in the Sabah Eco-Challenge; the Segama River was identified as the main source of contamination. In addition, an outbreak linked to swimming in a stream close to an oil palm

plantation in Kampung, Kebatu, Beaufort, Sabah, resulted in the identification of 46 cases from a few human breakouts. Finally, an outbreak of severe febrile illness was said to have suddenly broken out in the southern state of Johor as a result of intense rain and flooding. Pathogenic bacteria of the genus *Leptospira*, which are spirochetes that are a part of the order Spirochetales and family Leptospiraceae, are the source of leptospirosis. Leptospirosis is thought to cause 10 million cases annually, with a death rate ranging from 5% to 25%. Leptospirosis is manageable, though, provided it is identified and treated quickly. However, symptoms, laboratory testing, and epidemiological variables all relevant in the context of early diagnosis and therapy. A number of diagnostic techniques for this illness have been reported, including molecular diagnosis, microscopic demonstration, leptospire isolation and identification, and serological diagnosis employing techniques like the microscopic agglutination test (MAT). Because a large panel of live leptospires is required, this procedure requires a lot of work and time to execute. Some disadvantages of MAT are false-negative results obtained early in the course of the disease and cross-reactivity, which prevents serovar differentiation. This makes it difficult to administer, interpret, and score the MAT. Two serological methods, the micro-capsular agglutination test (MCAT) and the enzyme-linked immunosorbent assay (ELISA), reduce false-positives due to vaccine reactions and offer early identification. The IgM ELISA test has been shown to have an incredibly high sensitivity (100%) for identifying acute leptospirosis in Australia. When admission serum was used in Laos, the same commercial test, however, had poor diagnostic performance (sensitivity of 60.9% and specificity of 65.6%). Competent staff members can employ MAT, ELISA, and bacterial culture in well-equipped labs. Nevertheless, not many diagnostic centres have the necessary tools or expertise to carry out these leptospirosis tests. (see Figure 2)

**FIGURE 2:** Leptospirosis in South Africa

Correlation Coefficient

Table 2: Result of Correlation Coefficient

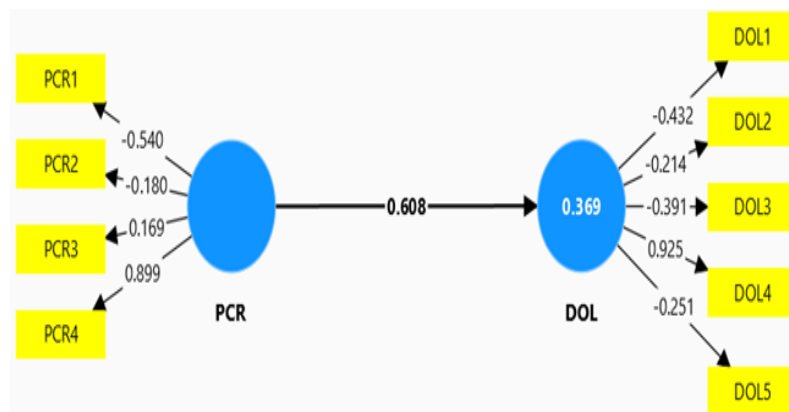
	PCR1	PCR2	PCR3	PCR4	DOL1	DOL2	DOL3	DOL4	DOL5
PCR1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PCR2	0.054	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PCR3	0.021	-0.509	1.000	0.000	0.000	0.000	0.000	0.000	0.000
PCR4	-0.120	-0.242	0.239	1.000	0.000	0.000	0.000	0.000	0.000
DOL1	0.123	0.110	-0.117	-0.116	1.000	0.000	0.000	0.000	0.000
DOL2	-0.259	0.086	-0.264	-0.196	0.163	1.000	0.000	0.000	0.000
DOL3	0.268	-0.021	0.205	-0.039	0.229	0.122	1.000	0.000	0.000
DOL4	-0.280	0.059	0.048	0.579	-0.201	-0.076	-0.130	1.000	0.000
DOL5	-0.080	-0.043	0.256	-0.170	0.027	0.163	0.135	-0.064	1.000

The above result shown in table 2 describes that correlation coefficient analysis result demonstrate that 0.054, 0.021, -0.120, 0.123 some negative and some positive correlation between independent and dependent variables. the DOL1,2,3,4 and 5 shows that 0.268, -0.280, -0.080, -0.021, 0.059, -0.043 some positive and some negative interrelation with each other.

Suggestion to Enhance Colombia's Leptospirosis Diagnosis

It was decided at the third conference of the Global Leptospirosis Environmental Action Network (GLEAN) in 2013 that real-time PCR might be a viable replacement for the accurate and fast diagnosis of leptospirosis. However, it was underlined that the cost-benefit ratio needs to be raised in order to promote early registration at each country's epidemiology center.

Smart PLS Algorithm Model

**FIGURE 3:** Smart PLS Algorithm Model

The above model shown in figure 3 represents that smart PLS Algorithm model between the PCR and DOL graph present that PCR shows -0.540, 0.180, 0.169, 0.899 its shows that 54%, 18%, 16% and

Real-time PCR is not yet commercially available in Colombia for the diagnosis of leptospirosis; nevertheless, "in-house" test costs should be less than COP 250,000 (USD 60.00). Its main advantage over other options for leptospirosis diagnosis is that it may be used to both tissue and blood samples. This enables quick findings and a precise and sensitive diagnosis during the acute stage of the illness as it can provide a positive result from blood before a detectable antibody titer has established. Depending on the primer design, it may be able to identify a large number of circulating species in the country without the need for matched serum samples. On the other hand, as of March 2023, the expected commercial cost of classical PCR in Colombia is COP 180,000 (USD 45.00). Its sensitivity is 100% (95% CI: 97.9–100%) in blood samples taken within the first seven days of the disease, while MAT's sensitivity is 71.1%.

89% significant relation between them. similarly, the Dol represent that -0.432, -0.214, -0.391, 0.925 also that -0.251 shows that negative and positive relation between them.

Model Estimation Analysis

Table 3: Result of Model Estimation Analysis

Factors	Saturated Model	Estimated Model
SRMR	0.150	0.150
d-ULS	1.019	1.019
D-G	0.278	0.278
Chi Square	70.072	70.072
NFI	0.148	0.148

The results above shown in table 3 indicate that the model is saturated, and the predicted model result indicates that the SRMR value is 0.150. Given that the chi square value is 70.072, the model is suitable for investigation. In a similar vein, the NFI score of 0.148 indicates a positive rate.

Conclusion

After an overview of these aspects of the implications of rapid PCR-based Tests for the detection of Leptospirosis in Rural areas, we can conclude that rapid PCR-based Tests are gaining much importance in healthcare sectors because of the Accuracy of results and compatibility with the environment. In conclusion, it was demonstrated that the mPCR was a potential addition to the early detection of leptospirosis. Targeting the LipL32 gene can also be an effective way to identify leptospiral infection since the mPCR's multiple sets of primers increase its sensitivity and specificity. The mPCR method demonstrated here should be a useful tool for the early identification of leptospirosis due to its excellent sensitivity and specificity, regardless of serovar. There is a local and national impact on the epidemiological surveillance system due to the underreporting of leptospirosis incidence. The economy is also impacted by the expense of hospital stays, the days that afflicted people are unable to work, and the years of lost output due to

early deaths. Not to add, patients who seek care from the hospital network for acute febrile condition without a known cause are becoming more resistant to microorganisms as a result of the abuse of antibiotics. Based on the information presented in this study, it is advised to utilize the PCR test as the national reference diagnostic for leptospirosis during the acute phase of the disease.

This test's speed, sensitivity, and specificity allow for early intervention and the collection of data that will improve the leptospirosis surveillance assessment criteria specifically, more precisely calibrated indicators of morbidity and diagnosis. It is easier to include the test in Colombia's surveillance laboratories due to the country's educated workforce and network of laboratories capable of performing the test; agreement on the test's validation and standardization standards would be the only thing needed to finish the procedure. The microscopic agglutination test (MAT) is recognized as an essential epidemiological reference serological test to identify the disease's causal serogroup. The combination of both tests PCR and MAT remains essential in order to increase diagnostic confirmation and knowledge about leptospirosis in Colombia, including crucial features like reservoirs, circulating strains, seasonal and geographic trends, and people at risk. This will enable the implementation of suitable preventive and intervention strategies.

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