

Lymphocytic Choriomeningitis Virus (LCMV): Current Status and Future Directions for Clinical and Molecular Diagnostic Techniques

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ABSTRACT

Lymphocytic choriomeningitis virus is an RNA virus that is often overlooked despite the potential to cause severe illness. It is a significant cause of viral meningitis, particularly in specific clinical situations. LCMV is transmitted to humans when they come into contact with the secretions of infected mice, and its strong neurotropism primarily results in neurological symptoms. The most vulnerable populations are fetuses and immunosuppressed individuals. LCMV infection acquired through various means can manifest with a wide range of clinical symptoms, varying from being asymptomatic to severe manifestations.

Additionally, in cases where individuals are affected by this viral infection, it can result in fatal central nervous system disorders. Specifically, in pregnant women, intrauterine LCMV infection has been observed to lead to fetal or neonatal mortality. Furthermore, it can cause chorioretinitis and hydrocephalus in infants, which not only causes significant harm but also results in long-term impairments.

Timely identification and immediate intervention are crucial in improving the prognosis, especially among high-risk groups and regions where the infection is prevalent. Failure to promptly diagnose the condition can lead to significant mortality rates and leave survivors with long-term neurological complications. Consequently, it is imperative to utilize the most appropriate laboratory diagnostic approach, considering the patient's clinical symptoms, exposure history to the virus, and the prevalence of the pathogen in the area, to facilitate accurate clinical detection. This comprehensive review encompasses various diagnostic methodologies employed in managing LCMV, encompassing clinical manifestations, diagnosis, treatment, and potential complications associated with viral infections affecting the central nervous system.

KEYWORDS: Diagnosis, Chronic infection, LCMV, CNS.

INTRODUCTION

Among the population, especially in developing countries, RNA viruses have a considerable prevalence and are responsible for many emerging infections¹. The arenavirus family encompasses the Lymphocytic Choriomeningitis Virus (LCMV), with

rodents as the primary transmission mode. This virus is frequently associated with human infections that can be acquired or congenital^{2,3}. Infections of the central nervous system (CNS) by viruses are a significant contributor to morbidity and mortality on a global scale, encompassing conditions such as meningitis, encephalitis, and meningoencephalitis⁴. After its discovery, LCMV was recognized as a primary cause of aseptic meningitis and neuroinvasive disease in the United States². The Armstrong strain of LCMV was initially isolated in 1933 from the cerebrospinal fluid of a woman who succumbed during the encephalitis epidemic in St. Louis, USA^{2,5,6}. Later on, it was confirmed that this strain plays a role in causing aseptic meningitis⁵.

The initial report of congenital LCMV infection surfaced in the early 1990s, and since then, several cases have been documented across Europe⁶. Patients who are diagnosed with congenital LCMV infection exhibit symptoms like chorioretinal degeneration, hydrocephalus, and long-term neurological abnormalities. Contrarily, most individuals who get infected with LCMV during childhood or adulthood experience moderate symptoms for a few

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weeks and eventually recover fully^{7,8}. However, prenatal infection can cause severe disease, leading to significant injury or permanent dysfunction⁹. While LCMV infection is not routinely screened for during pregnancy, maternal signs or fetal symptoms suggestive of infection in the context of exposure to rodents should trigger awareness among healthcare practitioners regarding the potential of LCMV infection¹⁰. When diagnosing the causative agent in patients with CNS infections, it is necessary to consider the most likely microorganisms, available diagnostic tests, and appropriate clinical specimens⁴. Understanding the epidemiology and clinical manifestations of particular infectious agents is crucial to identifying appropriate diagnostic approaches. Several serologic studies conducted in urban areas have shown that the prevalence of LCMV antibodies in human populations ranges from 2% to 5%¹¹. However, it's important to note that there is no available data about LCMV incidence and mortality rates specific to Pakistan. Globally, the virus demonstrates varying transmission patterns, with some regions experiencing sporadic outbreaks and others reporting more sustained cases. While the mortality rates associated with LCMV are generally low, severe cases can lead to fatalities. Continuous surveillance and research efforts are essential to understand the dynamics of LCMV better and to implement effective strategies for prevention and control globally and in regions with limited available data, such as Pakistan. Prompt detection plays a crucial role in effectively responding to an epidemic and enables the implementation of timely containment measures to minimize the possibility of amplification and potential international transmission. Although laboratory examinations, such as microbiological, immunological, and PCR methods, are widely available and reliable, they have limitations¹². The virus can be detected through various diagnostic techniques, whether directly or indirectly, such as emerging technologies based on fluorescence, assays based on the immune response, and approaches based on molecular methods. This article provides insights into the latest trends in diagnosing LCMV infection, a viral zoonotic disease.

Virology and genome structure

LCMV is categorized as a virus belonging to the Mammarenavirus genus within the *Arenaviridae* family, characterized by its negative-sense RNA. It is enveloped and can have a round, oval or pleomorphic shape with a diameter between 110-130 nm. The name 'arenavirus' comes from the sandy-appearing granules that resemble ribosomes present within the virus during morphogenesis, as seen through electron microscopy. The virus's genome consists of two RNA segments - S and L, each with an ambisense orientation. Four viral proteins, namely Nucleoprotein (NP), Envelope Precursor Glycoprotein (GPC) - which is divided into GP1 and GP2 subunits, Matrix Zinc-binding (Z) protein, and Large RNA-dependent RNA

polymerase (RdRp), are encoded by these RNA segments. Within the virion spike, the stable signal peptide (SSP) undergoes cleavage when the Envelope Precursor Glycoprotein (GPC) is synthesized. The Nucleoprotein (NP) is an essential structural protein for transcription and replication. Additionally, antigens present in GP1 are significant in neutralizing the virus¹³.

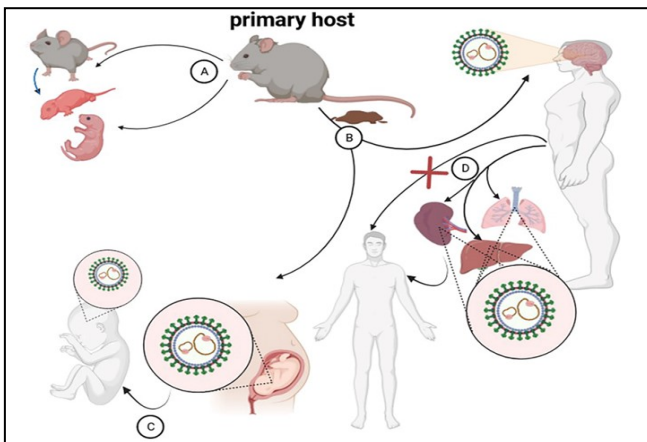
Pathogenesis

Attachment and replication of LCMV can occur in various cell types with α -dystroglycan (α -DAG1) as a cell surface receptor for extracellular matrix proteins^{14,15}. The muscles, neurons, heart, and brain contain the dystrophin-glycoprotein complex (DGC), which includes α -dystroglycan, a peripheral membrane component¹⁶. During infection, the virus engages receptors on the cell surface and is internalized through endocytosis. (α -DAG1) does LCMV use the primary cellular receptor for infecting cells. Following internalization, the process of transcription and replication of LCMV occurs within the cytoplasm. The S RNA segment of LCMV contains the negative-sense encoding for NP and the positive-sense encoding for GPC, which is later cleaved into GP1 and GP2.

On the contrary, the L RNA segment encodes RdRp in the negative sense and Z protein in the positive sense. The Z protein binds to the Ribonucleoprotein (RNP) complex. LCMV has a non-cytolytic life cycle, and the viral membrane is formed through bud formation from the plasma membrane, involving the incorporation of host lipids. Mutations in the glycoprotein (GP) of LCMV can decrease or eliminate the binding affinity to α -DAG1, leading to changes in viral persistence, kinetics, and cell tropism^{3,13}.

Reservoir, host and transmission

The house mouse (*M. domesticus*) serves as the primary natural host for LCMV. However, other rodents, such as yellow-necked mice, bank voles, rats, pet hamsters, and guinea pigs, can also transmit the virus to humans^{2,17}. LCMV is a prevalent virus globally but has a higher incidence in North America and Europe¹⁸⁻²⁰. Its occurrence typically peaks during the colder seasons (autumn and winter) as rodents migrate indoors, invading human habitats⁷. Vertical transmission allows the virus to persist across mouse generations, leading to chronic asymptomatic infections as these animals do not elicit an immune response^{21,22}. Transmission of LCMV can occur through various means, such as the inhalation or ingestion of infected rodent excreta, direct contact with rodents, and bites from them. While person-to-person transmission is infrequent, instances of viral transmission have been documented in solid organ transplant recipients and fetuses due to transplacental transmission^{23,24}. As a result, LCMV is classified as a zoonosis, wherein humans become infected after being exposed to infected secretions like nasal discharge, semen, saliva, urine, milk, or feces^{21,25}. (Figure I).

Figure I: Route of LCMV transmission

LCMV epidemiology is closely linked to the presence of a reservoir host and human interaction with rodents.

A: The primary mode of virus transmission occurs horizontally between rodents and via rodent bites. In addition, airborne transmission through contaminated aerosols from secretions is possible.

B: Humans can contract the virus by inhaling dust from waste or mouse secretions, consuming contaminated food or drink, or sustaining skin scratches.

C: Vertical transmission from mother to fetus is also possible with LCMV. The virus can be obtained from the blood and placenta of an aborted fetus.

D: It should be noted that LCMV does not spread horizontally from person to person; however, there have been reported cases of virus transmission through organ transplantation.

Classification

Significant diversity among LCMV strains has been revealed through phylogenetic analyses conducted on strains collected from various geographic and temporal sources. (3). Based on the S segment analysis, four primary lineages can be identified, with most LCMV strains found in lineages I-III associated with severe human diseases. Over 30 additional strains have been identified in humans and rodents across the USA, Europe, and Japan, exhibiting distinct tissue tropism patterns such as Armstrong (LCMV-Arm 53b), WE, Traub, Clone-13, Aggressive, and Docile²⁶. In 1933, Charles Armstrong was the first to isolate the original strain of LCMV-Armstrong from the brain. In 1935, the Traub strain was acquired from a laboratory colony of mice that were persistently infected². On the other hand, the WE strain was obtained in 1936 from an infected patient exposed to persistently infected mice. The WE strain causes aseptic meningitis more frequently in primates than the neurotropic Armstrong strain, which is viscerotropic in mice²⁷.

Significant variations in the virology and pathogenicity of LCMV strains used in laboratories make them helpful in studying different types of infections and host antiviral responses²⁸. For instance, Armstrong

and Clone-13 are two LCMV strains that differ slightly in their amino acid sequence, demonstrating that a few mutations can result in significant changes in viral biology. Armstrong causes acute infections and results in the natural elimination of the virus within two weeks, whereas Clone-13 replicates faster and creates persistent infections with detectable viremia for 90 days^{29,30}.

Clinical diagnosis (Signs and symptoms)

LCMV infection is identified by clinical signs and symptoms that vary depending on the patient's developmental stage. The clinical manifestations are determined by whether the infection occurs during the postnatal or prenatal period⁷. Although certain strains of LCMV are classified as "neurotropic" or "viscerotropic", all strains have the potential to replicate in the brain and cause various neurological symptoms^{25,31}. Approximately one-third of those whom LCMV infects will remain asymptomatic, while another third will have non-specific symptoms like fever, myalgia, or headache^{3,7}. In the final third, the CNS will be affected by more severe symptoms, mainly meningitis and meningoencephalitis³. Some clinical manifestations caused by infection with this virus include encephalitis, hydrocephalus, myelitis, pneumonitis, myocarditis, orchitis, parotitis, and increased CSF protein levels^{7,31}. The most susceptible hosts for the severity of LCMV infection are vulnerable immunocompetent individuals (adults and children), individuals with impaired immune systems (organ transplant recipients), pregnant women, and neonates^{7,32,33}. Also, the virus is more likely to be contracted by people who are involved in working with infected animals, such as pet store employees or farmers^{34,35}.

Therapeutic approach

Human LCMV infection has limited options for antiviral therapy. The current research focus on antiviral treatment for this disease is to repurpose drugs approved for treating other infectious diseases^{36,37}. Unfortunately, a vaccine or effective treatment for LCMV infection is not yet available. In severe cases of LCMV infection in humans, ribavirin, a guanosine analog, has been used as an initial antiviral drug. The drug can be taken either orally or intravenously and is considered a broad-spectrum antiviral with intricate mechanisms of action^{7,38}. These mechanisms encompass the inhibition of RdRp, the induction of mutagenesis, and the depletion of guanosine triphosphate. Additionally, ribavirin is known to function as an effective immunomodulatory drug, promoting the differentiation of naive CD4+ T-cells towards Th1-type cytokine responses that boost antiviral immunity. However, it is essential to highlight that ribavirin should not be taken by pregnant women³⁹.

Another antiviral drug, favipiravir, is a pyrazine-carboxamide derivative that interferes with the activity of RdRp in different RNA viruses. Despite its approval for clinical use against influenza in Japan, ongoing

clinical trials assess its effectiveness in treating COVID-19⁴⁰. The excellent antiviral effect of favipiravir, especially when administered early, has been demonstrated through studies utilizing murine models of acute disseminated LCMV infection and hemorrhagic disease⁴¹. While definitive treatment for LCMV infection remains unknown, certain antiviral drugs such as Favipiravir and Ribavirin have shown promising results against the virus⁴². Based on experimental studies, several compounds that interact with viral proteins and inhibit different stages of the replication cycle have been introduced, including Umifenovir (arbidol) and human monoclonal antibodies specific for glycoproteins of the Lassa virus^{43,44}. Despite these advances, no treatment is available for managing LCMV CNS infection; therefore, supportive care is the only available management option.

Methods for diagnosis of LCMV

Generally, two types of approaches for detecting viruses are direct and indirect. While the effects of the virus, such as cell death or the generation of antibodies by the infected person, are sought by indirect methods, the virus itself is examined by direct approaches. Among these methods, the following can be mentioned:

Indirect cell line-based methods

Virus culture

To cultivate and propagate most viruses, continuously growing cell lines are readily available⁴⁵. When virus particles attach to cells, morphological changes like cytopathic effects, cell death-induced plaques in the cell layer, inclusion bodies, and giant cells can be observed^{45,46}. These observations provide initial evidence of virus replication. In the shell vial assay, susceptible cells are exposed to suspected virus-containing material and subjected to low-speed centrifugation, resulting in an abbreviated virus culture. After 1-2 days of incubation, viral proteins can be detected through immunofluorescence or similar techniques⁴⁷.

LCMV can proliferate in diverse cell lines, such as BHK-21, Vero, and L-929 cells¹³. Diagnosis can also be accomplished by introducing blood or CSF via intracerebral inoculation into neonatal mice. However, due to LCMV's classification as a BSL-3 agent, all procedures must be conducted within a certified biosafety cabinet⁴⁷. Infected cells typically exhibit minimal to no cytopathic effect, making it difficult to differentiate between infected and uninfected cells. Using monoclonal antibodies in shell vial culture has improved speed and specificity⁴⁸. Nevertheless, CSF viral culture often cannot provide a timely diagnosis for optimal patient management because of its long time and low sensitivity⁴⁹. A plaque assay is necessary to determine if the virus was generated accurately.

Plaque assay

The naked-eye plaque counting technique can be employed to determine infectious viral titers. Various dilutions of the virus are absorbed into Vero e6 cells in

this process, leading to the development of plaques within five days⁴⁸. The Vero cell monolayers are subsequently fixed in agar overlay and are visualized through neutral red staining. Single infectious virus yields single plaques, which are prevented from merging with other plaques by the agar overlay⁴⁷. However, the sensitivity of the plaque assay is restricted by several factors, such as the utilization of tissue culture plates with a specific surface area, the incorporation of large assay volumes (up to 200 µl), and the ability of the human eye to differentiate individual plaques, this can be particularly challenging for LCMV due to its heterogeneity⁵⁰. While standard plaque assays have been historically used to quantify viral titers, this technique requires lengthy incubation times for non-cytopathic viruses like LCMV, making quick results unattainable⁴⁸.

Immunoassay detection-based method

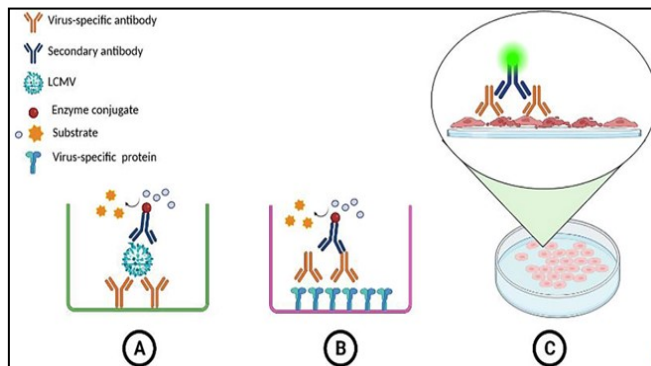
Antibodies against surface glycoproteins or glycoprotein (GP) on LCMV can neutralize the infection. Nevertheless, developing antibodies against internal antigens, such as the nucleoprotein (N or NP) responsible for packaging viral genomes, is also evident. Intriguingly, anti-N antibodies are generated earlier and in greater concentrations during infection than their anti-glycoprotein counterparts⁵¹. In acute Lymphocytic Choriomeningitis Virus (LCMV) infection, anti-N antibodies can be detected as early as four days, and their concentration remains elevated compared to anti-GP antibodies throughout the immune response⁵². Although LCMV is usually cleared within a few weeks, a neutralizing antibody concentration may only appear after several months and persistence of the virus^{53,54}; this suggests that antibodies are essential for resolving infection because in mice with restricted antibody specificity or lack functional B cells or an ability to produce soluble antibodies, viraemia continues for a month or more⁵¹. To definitively diagnose CNS infections serologically, it is necessary to detect IgM antibodies or establish evidence of a minimum fourfold rise in neutralizing antibody titers between CSF samples taken during the acute and convalescent phases^{49,55,56}. Because antibodies take time to develop after symptoms appear, a negative antibody test cannot exclude the possibility of infection, potentially necessitating retesting⁴⁵. These tests may not provide optimum sensitivity in specific populations, such as immunocompromised individuals^{49,55}. Nucleic acid amplification tests have replaced antibody-based detection as the preferred test in most situations; however, these assays still have a valuable role as a standard test for most infected individuals with LCMV⁴. Serology is the most common method for diagnosing LCMV based on protein detection⁴⁷. Enzyme-linked immunoassay (EIA) and Immunofluorescence assay (IFA) can quantify antibody responses in sera against viral antigens expressed in infected cells, detecting IgM and IgG antibodies¹³. Access to these tests, however, is limited

to a select number of laboratories. Diagnosis involves assessing increased antibody titers in acute and convalescent serum samples. Confirmation of the diagnosis can be achieved by detecting specific IgM in both blood and cerebrospinal fluid (CSF)⁵⁵. The Enzyme-linked immunosorbent assay (ELISA) has been developed, offering a rapid, straightforward, and effective means for clinically detecting anti-nucleoprotein antibodies in human sera (**Figure II**).

Immunofluorescence focus assay (IFA)

In the modified version of the conventional plaque assay, known as the IFA (Immunofluorescence Assay), the primary distinction lies in utilizing a rat anti-LCMV monoclonal antibody VL4. This antibody, which has an affinity for the nucleoprotein of LCMV, is employed, thus enabling binding in infected cells^{55,56}. LCMV titer can be measured using this antibody occasionally, and visualization can be achieved by employing a fluorescently tagged secondary antibody. The IFA offers certain benefits over the plaque assay, such as enabling more straightforward and efficient virus quantification with higher throughput⁷. Furthermore, results can be obtained within a day through IFA, whereas the plaque assay requires five days for completion⁴⁷. However, one drawback of the IFA is that it solely detects viral antigens that have been assimilated by the cell and does not gauge the presence of infectious viruses⁵⁶.

Figure II:
Reaction processes of different ELISA types



A: An antigen-capture ELISA is utilized to detect viral proteins or particles. The process begins with virus particles from the sample material binding to virus-specific antibodies coated on the well walls, followed by the addition of enzyme-conjugated antibodies against a different domain of the viral particle. The final step involves substrate addition; if successful, the enzyme transforms into a colorful molecule.

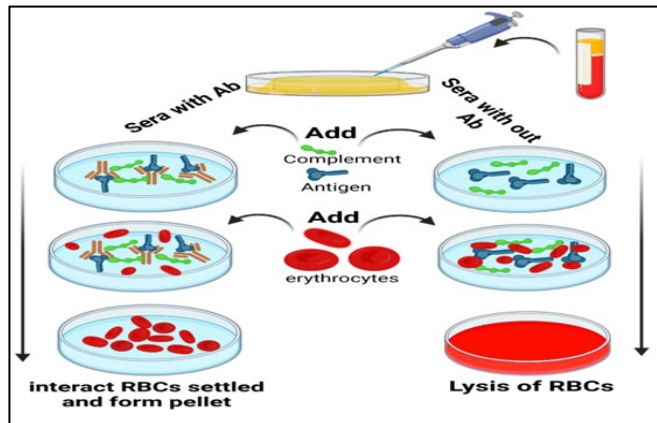
B: Employs ELISA to identify specific antibodies. This method is similar to the previous one except that virus-specific protein is coated on the well walls instead of virus-specific antibodies. In favourable situations, patient serum contains antibodies against the viral protein, and enzyme-conjugated secondary antibodies against the Fc region of patient immunoglobulins are used for antibody capture.

C: Involves several reaction steps in the immunofluorescence test.

Complement fixation test

The complement fixation test (CFT) represents a conventional immunological laboratory diagnostic assay that remains in use for detecting virus antigens or antibodies in patient sera or cerebrospinal fluid samples during an acute infection. In this procedure, the patient's serum undergoes heat treatment to initially inactivate the complement system, following which it is combined with the viral antigen. Subsequently, an exogenous complement (typically sourced from fresh guinea pig serum) is introduced once antigen-antibody complexes have formed and are then incubated⁵⁸. The tests are usually performed on microtiter plates and visually observed⁵⁸. Although CFT is utilized to diagnose acute viral infections, it is unsuitable for investigating the host immune status underlying LCMV. The assay procedure for the CFT is complex as it relies on numerous biological variables that need to be standardized through pretesting. This method is less sensitive than other immunoassays and involves a lot of manual work, making it unsuitable for automation⁵⁹. Due to significant limitations such as being time-consuming, labour-intensive, and often non-specific (cross-reactivity), the utilization of CFT in LCMV virus diagnostics is gradually being replaced by modern immunoassays⁵⁹. (**Figure III**).

Figure III: Complement Fixation Test Principle



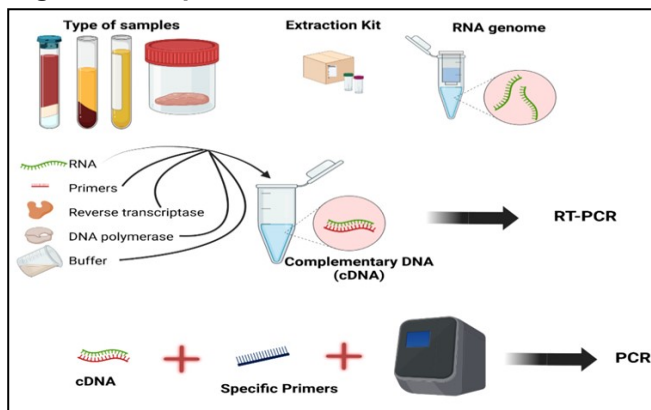
When a sample already contains the intended antibodies or antigens, an Ag-Ab complex will form upon adding a complementary reactant. The type of reactant utilized depends on the component being detected. As a result of the Ag-Ab complex formation, the indicator system cannot interact with the added complement, and no change in the indicator system is observed. In contrast, if the intended antibodies or antigens are not present in the sample, the Ag-Ab complex will not form, and the complement will affect the hemolysis of red blood cells used in the indicator system.

Nucleic acid-based detection assays

Usually, the preferred detection method has become

nucleic acid amplification tests rather than antibody-based tests^{4,60}. Significant advancements have been made in molecular biology techniques, and various laboratory techniques based on molecular approaches, such as polymerase chain reaction (PCR) and real-time PCR, are commonly employed to identify multiple viral species^{47,61}. PCR can detect viral RNA in serum or cerebrospinal fluid (CSF), while quantitative PCR is a more sensitive technique that can quantify LCMV^{48,49}. However, it may detect residual nucleic acid that remains present for several days after the controlled viral antigen, which cannot indicate an active infection. Real-time PCR enables the monitoring of PCR amplification along with the original assay's specificity and sensitivity⁶⁰. This technique uses various fluorescent agents, including TaqMan probes, SYBR Green dye, and Scorpion primers, to quantify the sample^{62,63}. Similarly, the development of reverse transcription polymerase chain reaction (RT-PCR) has facilitated the detection of LCMV RNA in blood and CSF. The GPC and N genes are targeted by highly sensitive assays⁶⁴. Techniques for enriching viral genomes through nucleic acid amplification include the use of rolling circle amplification for viruses with a circular genome, the employment of restriction enzyme sites that are more abundant in viral nucleic acids compared to humans, followed by adaptor ligation and PCR amplification, and the utilization of specific oligonucleotides that obstruct the reverse transcription and amplification of host nucleic acids (such as rRNA)^{65,66}. **(Figure IV).**

Figure IV: Steps involved in RT-PCR



This figure outlines the steps involved in RT-PCR, which entails gene extraction from various samples, including whole blood, serum, CSF, and infected tissues. This approach utilizes reverse transcription to convert RNA into complementary DNA, which is subsequently amplified using the polymerase chain reaction (PCR) to target specific DNA sequences. The primary objective of this method is to measure the quantity of RNA, which can be achieved by using real-time PCR or quantitative PCR (qPCR) that employs fluorescence to monitor the amplification reaction. The

combination of RT-PCR and qPCR is widely used in research and clinical settings for viral RNA quantification and gene expression analysis.

Future direction for diagnostic methods

Next-Generation Sequencing (NGS)

NGS has proven to be a successful diagnostic tool in virology, particularly in cases where conventional diagnostic methods cannot detect viral pathogens. The organism is not only identified but genotypic markers of drug resistance and virulence, as well as strain typing, are also detected by NGS methods^{67,68}. Next-generation sequencing has also been applied to donor-derived LCMV infections⁶⁹. Several clinical fields have validated and utilized NGS approaches to identify and characterize medically significant pathogens. However, rigorous validation and comparison of NGS methods and bioinformatics pipelines with traditional diagnostic techniques are necessary to identify and characterize pathogens^{67,70} accurately. Despite its advantages, widespread implementation of NGS in clinical microbiology laboratories requires expensive new equipment and personnel trained in bioinformatics-reliant techniques. In this context, selecting appropriate bioinformatics tools is critical to the success of viral discovery. In amplicon sequencing experiments where the reference genome is known, read alignment software utilizes strict mismatch rules to minimize errors^{67,71}. However, if the target virus is highly divergent and not present in publicly available viral databases, it may be impossible to map reads. In such cases, reads must be assembled into contiguous sequences by bioinformatics tools, which identify overlapping sequences between them. Bioinformatics pipelines should provide user-friendly interfaces to allow data input directly from sequencing instruments⁷². Moreover, these tools should provide the best match hits to comprehensive and well-curated reference genome databases^{67,72}.

Other suggested diagnostic methods.

A relatively DNA or RNA-based method called microarray has proven to help locate and classify viruses by attaching a variety of known nucleic acid fragments, ranging from thousands to millions, to a solid surface termed a "chip". Next, the RNA or DNA extracted from the study sample was applied to the chip⁷³. DNA microarrays are used to identify or quantify specific DNA sequences in complicated nucleic acid samples⁷⁴. The genomic makeup of viruses can be targeted using DNA microarrays. Depending on the application, the design may change; however, the fundamental procedure is to separate the RNA from a cell sample, apply reverse transcriptase PCR, and fluorescently label the nucleic acid product. Following this, various immobilized oligonucleotides specially designed for the genetic makeup of the virus of interest were screened using the fluorescently labeled nucleic acids produced. Some viruses have been successfully identified and genotyped using microarray⁷⁵⁻⁷⁷. CRISPR-C

systems have been developed to protect bacteria from bacteriophages and other foreign nucleic acids, but they have revolutionized our ability to edit genes and control gene expression⁷⁸. Effectors of types III and VI with RNA-targeting activity defend against RNA infections, whereas DNA-targeting effectors such as Cas9 offer defence against invading DNA bacteriophages. The class 2 type VI CRISPR effector Cas13 has recently received attention because of its potent ability to target and cleave RNA in various model systems, including mammalian cells⁷⁹⁻⁸¹. Additionally, Cas13 can analyze CRISPR arrays, which contain and release distinct CRISPR RNAs (crRNAs), enabling multiplexed targeting applications (82). In addition to its ability to process CRISPR arrays, Cas13 exhibits collateral cleavage activity, which has been used in diagnostic procedures, such as specialized high-sensitivity enzymatic reporter unlocking (SHERLOCK)⁸²⁻⁸⁴. They seem to be using Cas13's ability to target and modify RNA in a new way. They call it the "Cas13-assisted can be used to restrict viral expression and readout" or CARVER technology. This technology combines Cas13 to cut the viral RNA and quickly check it using the SHERLOCK platform for diagnosis^{84,86}. According to numerous recent studies, aptamers can be used as diagnostic or therapeutic tools for viral infections⁸⁷. Aptamers are DNA or RNA molecules chosen *in vitro*, are highly selective, and can bind various nucleic and non-nucleic acid molecules⁸⁸. Aptamers compete with monoclonal antibodies because of their unique properties, which enable them to identify virus-infected cells or viruses directly. Each step of the viral replication cycle can be disrupted using specific aptamers, preventing the virus from entering the cells⁸⁹.

Virus detection in congenital infection

Clinicians should be knowledgeable about congenital LCMV infection and its significance. In cases where chorioretinitis and fetal hydrocephalus remain unresolved, it is crucial to consider the possibility of congenital LCMV infection⁹⁰. Exposure to rodents of the mother during pregnancy is a risk factor that significantly raises the chances of contracting LCMV infection and subsequent transplacental transmission of the virus⁹. A definitive diagnosis can be made by identifying the virus through serological analysis or direct evidence, such as discovering the virus through isolation or detecting LCMV RNA in fetal or maternal samples^{9,10}. A positive PCR result and sequenced confirmation are considered direct evidence of the presence of LCMV^{7,91}. Comprehensive ultrasonography may be performed when ultrasonographic indicators of infection are present to detect potential abnormalities associated with conventional congenital infections³². If the initial assessments yield negative results, testing for LCMV is necessary on fetal and maternal serum samples³². The diagnosis of congenital LCMV infection in infants can be challenging. Most babies with this condition do

not carry the infectious virus at birth⁹. Therefore, the diagnosis should be confirmed through serological tests, which are further complicated by transplacentally transferred maternal IgG antibodies. As a result, serologic assessments for LCMV need to comprise both IgM and IgG titers on both infant and maternal serum samples^{9,90}. Although the indirect immunofluorescence assay has been utilized to validate the diagnosis, other techniques, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), have also helped diagnose LCMV.

LCMV detection in organ transplant conditions

Concerns have arisen regarding the transmission of LCMV through infected solid organ transplants, particularly for immunocompromised individuals⁹². Until April 2013, six clusters of organ transplant-associated LCMV and LCMV-like arenavirus transmissions had been reported in the USA and Australia. These clusters included recipients of kidney, liver, lung, and cornea transplants^{23,69,93}. Diagnosing LCMV in the transplant population requires a combination of testing methods; this includes detecting LCMV-specific IgM/IgG in cerebrospinal fluid (CSF) and serum and identifying LCMV through RT-PCR or virus isolation from CSF, serum, and tissues⁵⁶. In cases where serology is negative, antigen detection using immunohistochemical staining in tissue specimens can be useful^{23,56}. Per serology and RT-PCR testing on serum and CSF can improve diagnostic accuracy. Interestingly, even with RT-PCR or serology testing, LCMV may not always be detected in donors. In this regard, further investigation and alternative testing methods should be considered. Exploring additional diagnostic approaches or conducting repeat tests is essential to enhance detection sensitivity. Additionally, thoroughly screening donors' medical histories and potential risk factors may provide valuable information. If there are persistent concerns about LCMV transmission, consultation with infectious disease experts and healthcare professionals can help determine the most appropriate course of action for ensuring the safety of the blood supply or relevant biological samples^{33,94}. The most commonly utilized serological tests for detecting specific anti-LCMV IgM or IgG antibodies are ELISA and indirect immunofluorescence assay (IFA)⁶⁰. The complement fixation test is insensitive and should not be used⁵⁹.

CONCLUSION

Identifying viral agents in patients with CNS infections has been difficult due to various factors, including low CSF culture yield and delayed organism-specific antibody production. Furthermore, difficulties in sample collection have added to the challenge. However, nucleic acid amplification-based molecular diagnostic methods have revolutionized clinical microbiology practices. They provide a more sensitive, specific, and convenient approach to diagnostic

testing. Although these methods have advantages, positive results can lead to detecting viruses that are not actively replicating and, therefore, do not contribute to the pathogenesis. Researchers are exploring adjunctive biomarkers indicating active replication to overcome this issue. Developing cost-effective, sensitive, and specific molecular diagnostic methods has significantly improved the recognition of congenital LCMV infection, which was previously considered rare. This progress highlights the constant need for improved diagnostic techniques and emphasizes the potential benefits of investing in research to create more accurate and effective diagnostic tools.

Disclaimer: This article contains no studies performed by authors with human participants or animals.

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AUTHOR CONTRIBUTION

Eslami N:	Writing original draft
Jahanabadi S:	Writing original draft
Ziaei E:	Data collection
Shenagari M:	Data collection
Salmanzadeh S:	Data collection
Abbasi S:	Supervision of research
Zandi M:	Conceptualization of research

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