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# A Novel Latex Agglutination Diagnostic Technique for Rapid Detection of Rift Valley Fever Virus Antibodies

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**Abstract**

RIFT VALLEY FEVER (RVF) is a zoonotic illness that poses a substantial public health risk in certain regions of Africa and the Arabian Peninsula. It affects both humans and ruminants. Timely and precise identification of viruses during outbreaks is essential for implementing successful management methods. This study focused on the development and assessment of a new diagnostic method that utilizes latex agglutination to detect antibodies against the whole Rift Valley fever (RVF) virus. The results have shown a high level of diagnostic accuracy, with a rate of 91.2%. The test demonstrates a sensitivity of 92.59% in identifying positive serum samples, as well as a flawless specificity rate of 100%. This approach, which is simple, fast, and inexpensive, has significant potential for screening animals for RVF on a wide scale. The discovery outlined in this study could have substantial ramifications for managing the dissemination of RVF and reducing its effects on the health of animals and humans.

**Keywords:** Rift Valley fever, Zoonotic disease, Virus detection, Latex agglutination, Public health concern, Diagnostic technique.

## Introduction

Rift Valley fever (RVF) is a sudden viral disease transmitted by arthropods that can lead to serious illness in domestic animals such as buffalo, camels, cattle, goats, and sheep, It is a crucial zoonotic disease [1]. Severe clinical indications are typically observed in cattle and small ruminants, where it is characterized by a high fever of 41 degrees Celsius. Abortions and a significant neonatal mortality rate [2].

In older lambs and mature sheep, the disease can manifest as either a per acute form, characterized by sudden death without prominent signs, or an acute form, characterized by fever lasting 1-3 days followed by death approximately three days after infection [3].

RVF is a significant public health concern disease, With its first detection outside of Africa, the illness made its way to the Arabian Peninsula in the year 2000. This sparked fears that the Rift Valley fever could spread to other regions of Europe and Asia. [4,5].

Several factors contribute to the spread of RVF in these areas, including competent mosquito vectors, a diverse range of susceptible domestic and wild animals, and climate change affecting non-endemic regions. The RVF virus (RVFV) belongs to the Phlebovirus genus within the Bunyaviridae family. Like other members of this family, RVFV is an enveloped virus with a single-stranded tripartite RNA genome consisting of large (L), medium (M), and small (S) segments [6].

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RVFV is primarily transmitted among ruminants through direct contact with infected animals' bodily fluids and mosquito bites, primarily from Aedes and Culex genera species. Additional hematophagous flies have also been shown to transmit the virus through biological or mechanical means in experimental settings, although their field applicability remains unknown [7,8] .In humans, the most common modes of transmission include direct contact with blood, consumption of raw milk, ingestion of infected animal excrement, and, rarely, mosquito bites [9].

The virus was first discovered in Kenya in 1930 and has since been prevalent in sub-Saharan Africa, The appearance of the Rift Valley fever virus (RVFV) in new regions was documented during outbreaks in Egypt in 1977 [10,11]. The initial occurrence of RVF outside

Mainland Africa was observed in Mauritania and Senegal in 1987 [12]. Subsequently, it spread to Saudi Arabia and Yemen in 2000 [13], Comoros in 2007[14],and Madagascar in 1990 [15]. South Africa has experienced three significant RVF epidemics, documented in the literature as occurring in 1950-1951, 1974-1975, and more recently, from 2008-2011. The recent outbreaks of RVF in Mayotte, Niger, Uganda, and Sudan have had fatalities among humans and have been characterized by widespread fetal loss and increased mortality rates among young ruminants [16, 17].

Efficient management of disease outbreaks relies on prompt identification of clinical signs by farmers, timely diagnostics through laboratory tests, and swift notification to relevant veterinary authorities. The faster these measures are implemented, the better the disease management outcomes. Accurate results require the submission of specimens to a referral lab to detect the presence of RVFV, which is characterized by a high viral load in the serum during the acute phase of the disease.

Various established procedures have been utilized to identify RVF, including virus isolation, determination of specific IgM or IgG antibodies, and detection of reliable nucleic acids associated with RVFV. One widely used technique for serological diagnosis of RVF involves the utilization of enzyme-linked immunosorbent assays (ELISA), which rely on either recombinant nucleocapsid protein N or whole virus antigens, as supported by prior research [18,19].

The primary approaches for rapid detection and quantification of RVFV during outbreaks include both traditional and real-time RT-PCR assays, which have shown remarkable sensitivity [20].However, advanced technologies such as next-generation sequencing (NGS) methods and colorimetric-based techniques, as well as TaqMan array cards, have emerged as alternative strategies [21,22].

It is important to note that implementing these methods can be prohibitively expensive and require highly skilled individuals' involvement and biosafety level three laboratories, which are not widely available in regions where the disease is prevalent.

Using an inexpensive and straightforward slide agglutination assay to identify specific viral etiologies associated with infections in a given area holds great promise for improving vaccination programs. This approach allows for targeting prevalent serotypes while avoiding unnecessary immunization with less prevalent serotypes. However, this assay can be competently performed by veterinary personnel with minimal training, saving time and resources. Additionally, it helps overcome the logistical challenges associated with transporting samples at low temperatures [23].This study aimed to develop and evaluate latex agglutination test(LAT) for RFV. equilibrated at room temperature before their use in the experiment.

## Material and Methods

*Virus strain and its culture and titration*

The virulent ZH-501 strain of RVFV was purchased from the Veterinary Serum and Vaccine Research Institute in Abbassia, Cairo. The viral specimen was cultivated in Hank's solution and introduced into a fully-grown baby hamster kidney (BHK) cell culture. The cell specimens were cultivated and sustained following the methodology outlined by Macpherson and Stocker [24]. Before experimentation, the cell cultures underwent screening for bovine viral diarrhoea virus (BVDV) and mycoplasma contamination. The virus was allowed to undergo adsorption by subjecting the cell cultures to incubation at 37$^{o}$C for one hour, with 10 $\mu$l of the virus added to each well and three wells for each dilution. Subsequently, maintenance media was added, and the cultures were re-incubated at 37$^{o}$ C. Cytopathic effects (CPE) were observed daily for 5-7 days. The titer of the virus was quantified as

TCID50/0.1ml of the initial inoculum, using the formula established by Reed and Munich in 1938 [25]. The determined titer of the RVFV was 107.5 TCID50.

*Lab animal inoculation*

All experimental animal procedures were conducted in compliance with the protocols approved by the Animal Ethics committee (AEC), under the application number (Vet CU 08072023675), and sponsored by the Faculty of Veterinary Medicine, Cairo University. Swiss albino-weaned mice aged 21-30 days were used for virus titration. The virus was diluted in a serial tenfold manner, and each dilution was administered to ten adult mice. A control group of uninfected mice was also included for comparison. Intraperitoneal (IP) injections of 0.1 ml of the virus were administered to each mouse daily for ten days, starting on the second day of inoculation. Any mice that expired within the first 24 hours were excluded from the analysis as non-unique mortality events. Following the above mentioned -formula, the virus titer was quantified as log10 MIPLD50/0.1 ml of the authentic inoculum.

*Serum samples*

a total of 82 blood samples were collected from non- vaccinated and 81 vaccinated sheep and goats that received the RVF vaccine. These samples were obtained from exceptional governorates of Egypt, specifically Maras Matrouh, Minya, and Halayeb w Shalaten. Upon collection, the blood samples were processed to separate the serum. The sera were then inactivated at 56$^{o}$ C for thirty minutes and stored at -80$^{o}$ C until further use in the serum neutralization test (SNT). All reagents used in the SNT were

*Latex beads*

An aqueous suspension of carboxylated modified polystyrene with an average particle length of 0.9 mm and a white color was obtained from Sigma-Aldrich under product number CLB9. The 300 $\mu$l beads were suspended in a 15 ml solution of 0.05 M glycine saline. The beads were then subjected to centrifugation for 15 minutes at 12,500g. The procedure above was iterated to collect the beads.

*Latex agglutination test (LAT)*

RVFV was applied to latex beads using the method described previously in Hay's 2002 [25].The inactivated virus was combined with a 10% (w/v) suspension of latex beads prepared as previously described. The resulting mixture was then added to 2.5 ml of glycine saline and incubated at 4$^{o}$C for 8 hours to achieve binding of the latex protein. Subsequently, the beads were recovered by centrifuging the suspension for 15 minutes at 12,500g. A second wash was carried out on the beads with 2.5 ml of 0.05 M glycine saline solution, and the resultant mixture was then subjected to 12,500g centrifugation for 15 min to collect the beads. Subsequently, the beads were reconstituted in 2.5 ml of 0.25 M glycine saline solution and preserved at 4 C for further use.

*In-field latex agglutination test (LAT)*

The virus-coated latex beads were used to test all serum samples. A 10 $\mu$l volume of each serum sample and inactivated virus was applied to latex beads coated directly onto a clean glass slide in equal volumes. The glass slide was free of grease. The serum sample and latex beads were mixed homogeneously using a wooden toothpick and assessed for agglutination, as evidenced by clumping within one minute (illustrated in Figure 1). The category of samples was based on the degree of agglutination observed and included negative, weak positive, positive, and strong positive classifications

*Serum neutralization test (SNT)*

The serum samples underwent testing to detect the presence of antibodies specific to RVF using the SNT. The test procedure adhered to the guidelines outlined in the OIE Manual of OIE 2015 [26]. Serum samples were subjected to heat inactivation at 56$^{o}$C for 30 minutes. Subsequently, 25 $\mu$L of cell culture medium containing 5% negative RVF serum and antibiotics were introduced into each well of a 96-well cell culture plate. The primary well of each row received 25 $\mu$L of serum, which was then diluted tenfold using a multichannel pipette. Duplicate titrations were performed on each serum sample, ranging from 1/10 to 1/640, to determine the endpoint titers. To ensure accuracy, manipulated sera of known positivity and negativity were included. Additionally, 25 $\mu$L of RVFV, diluted in conventional medium to achieve a concentration of 100 TCID50 per well, were added to each well containing diluted serum and to the wells containing positive and negative control serum. The plate was incubated for 30 minutes. Subsequently, 50 $\mu$L of BHK cells, appropriately diluted to yield a confluent monolayer within 12 hours, were added to each well. The plates were placed in a $CO_2$ incubator for approximately three to five days. Daily, the monolayers were examined under an inverted

microscope to detect any cytopathic effects (CPE). Rows containing positive control serum displayed no CPE, while rows containing negative control serum exhibited clear indications of CPE, confirming the virus's presence. The results were determined by calculating the reciprocal of the lowest dilution that yielded positive results. It is worth noting that a minimum positive titer of RVF- neutralizing antibodies (>40) has been recommended.

*Assessment of diagnostic and analytical sensitivity (DSe/ASe) and diagnostic and analytical specificity (DSp/Asp) following OIE guidelines*

In the validation process of an assay, the main performance index involves the estimation of DSe, the ratio of confirmed infected animal samples that are confirmed as positive by the assay, and DSp, the ratio of confirmed uninfected animal samples that are confirmed as negative by the assay. Analytical specificity (ASp) is the capability provided by the test to discriminate between target analytes, such as a viral or bacterial antigen, and no target analytes, such as matrix components. The analytical sensitivity (ASe) refers to the amount of target analytes predicted to be present in a matrix that will yield a positive result in at least a certain percentage of cases and is also relevant to assay validation. All statistical analyses were done using R and SPSS.

## Results

Serum samples from both vaccinated and unvaccinated ovine and caprine populations were subjected to the SNT, the established benchmark for RVF diagnosis. Comparative analysis was performed with the LAT to ascertain its diagnostic specificity and sensitivity. The LAT detected a spectrum of antibody responses in serum from sheep and goats immunized with a locally produced inactivated RVF vaccine, ranging from weakly positive to strongly positive, as verified by a positive control (Fig. 2). This finding underscores the LAT's capacity to identify RVF antibodies in vaccinated subjects.

The concordance between the SNT titers and LAT responses in serum samples from vaccinated sheep and goats is depicted in Table 2. The LAT responses demonstrated a symmetrical distribution when compared to the SNT results, adhering to the standards prescribed by the World Organization for Animal Health (OIE). The LAT effectively mirrored the gradation of SNT titers, which spanned weakly positive to strongly positive.

The diagnostic sensitivity of the LAT was assessed using 81 seropositive samples for RVFV as confirmed by SNT. Six samples tested negative by LAT, yielding a diagnostic sensitivity of 92.59% (Table 3). This high sensitivity rate underpins the efficacy of the LAT in detecting an RVF immune response in vaccinated cohorts.

To gauge the LAT's specificity, random serum samples from unvaccinated sheep and goats were evaluated, with Fig. 3 depicting exclusively negative reactions, affirming the absence of RVF antibodies. Furthermore, specificity was quantified by analyzing field samples from unvaccinated animals with both LAT and SNT (Table 3). All 82 samples tested negative in both assays, culminating in a specificity rate of 100%, thereby reinforcing the LAT's precision in identifying uninfected animals.

In summary, the LAT demonstrated a diagnostic accuracy of 91.2%, with an impressive area under the receiver operating characteristic curve of 0.963 and a specificity of 100% (Table 4 and 5), when benchmarked against the gold standard SNT for RVF diagnosis in sheep and goats. The consistency between LAT and SNT outcomes validates the reliability of LAT as a swift diagnostic tool for RVF outbreaks and surveillance purposes in settings with limited resources.

## Discussion

The use of latex beads in medical diagnostics dates back to its initial application for identifying rheumatoid factors by Plotz and Singer[32].Since then, latex-based assays have been refined to detect a variety of infectious diseases, autoimmune disorders, hormones, drugs, and antitoxins [33]. One such application is the detection of RVF infection. A key focus of this research is to develop an affordable, straightforward test for RVF using serum samples and latex agglutination (LAT), and to evaluate the test's sensitivity and specificity against the gold standard SNT.

In this study, we collected 82 blood samples from sheep and goats that had not been vaccinated against RVF, as well as 81

samples from vaccinated animals, across several Egyptian governorates (Marsa Matrouh, Minya, and Halayeb w Shalaten). We assessed the LAT's diagnostic sensitivity using 81 serum samples confirmed to be positive for RVFV via serum neutralization. Six samples tested negative, yielding a diagnostic sensitivity of 92.5%, slightly lower than the SNT's 100% sensitivity. On testing 82 known negative serum samples for RVFV, the LAT correctly identified all as negative, resulting in a diagnostic specificity of 100%, with the SNT again demonstrating 100% specificity. A comparison of our LAT with other published tests for RVF is presented in Table 5. The results indicate that while the SNT maintains superior diagnostic sensitivity, the LAT is sufficiently reliable for initial RVF outbreak detection or monitoring within disease control programs.

The implementation of LAT as a primary diagnostic tool could significantly improve the speed of initiating epidemiological responses to RVF outbreaks. Additionally, the development of a stable, non-infectious standard for transporting diagnostic samples is imperative, given the logistical challenges and associated costs. The expense of the LAT and its distribution to regional centres must be weighed against potential savings from reduced standard sample collection and transport costs. Market forces and competitive products from private firms must be factored into considerations for sustainable and affordable long-term use in regions with limited resources.

## Conclusion

Using LAT for diagnosing RVF outbreaks shows promising results regarding sensitivity and specificity. While the gold standard test (SNT) still outperforms the LAT, the latter provides a cost-effective and easy-to-use alternative for rapid diagnosis and disease surveillance. Further validation and consideration of logistical factors are needed before widespread implementation.

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This study didn't receive any funding support.

*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

**TABLE 1. Sensitivity and specificity of different serological and molecular tests available for detecting RVF antibodies .**

| Governorate | Diagnostic Sensitivity | Diagnostic Specificity | References |
|---|---|---|---|
| **Indirect ELISA** | 85.3% (65.5–99.1%) | 98.6% (97.1–99.8%) | [27]. |
| **C ELISA** | 95.1% (83.5-99.4%) | 91.8% (85.0-96.2%) | [28]. |
| **Real-time PCR** | 97.6% (93.9–99.8%) | 99.4% (96.9–100%) | [29]. |
| **PRNT80 [33]** | 84.4% (66%–97.3%) | 98.1% (95% CI- 99.6%) | [30]. |
| **LFT [34]** | 100% | 98.8% | [31]. |
| **Our study LAT assay** | 92.593% (84.571% to 97.233%) | 100.000% (95.601% to 100.000%) | |

**TABLE 2. Comparison of SNT Titer and Latex Agglutination Test Results for Serum Samples from Vaccinated Sheep and Goats with Local Inactivated RVF Vaccine. The results demonstrate the correlation between the results of both LAT and SNT. The SNT titers are categorized as follows, based on OIE guidelines. The table showcases the symmetry between the results obtained from LAT and SNTfor the vaccinated animals**.

| Sample | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| **LAT** | Weak positive | Weak positive | Positive | Positive | Strong positive | Strong positive |
| **SNT titer** | 80 | 80 | 160 | 160 | 640 | 640 |

SNT titers are categorized as follows, based on OIE guide- lines: 80 (weak positive), 160 (positive), and 640 (strong positive). The table showcases the symmetry between the results obtained from LAT and SNTfor the vaccinated animals.

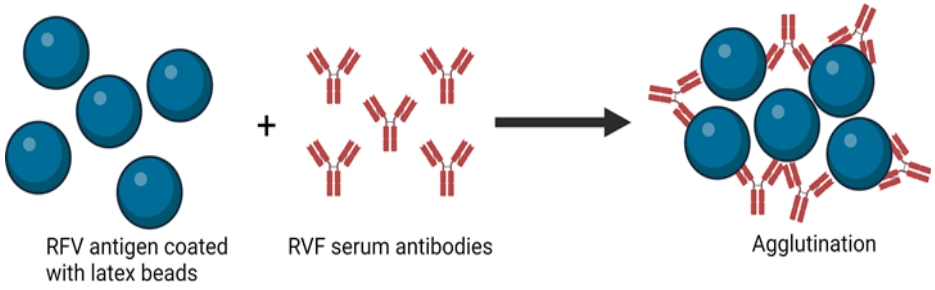


**Fig. 1. Schematic representation of the latex agglutination assay for rift valley fever**

**TABLE 3. Sensitivity and Specificity of Latex Agglutination Test Compared to Serum Neutralization Test for RVF Diagnosis in Vaccinated and Non-Vaccinated Sheep and Goats Vaccinated with Local RVF vaccine**

| Governorate | Total Samples | Vaccination Time | Sampling Date | Vaccination Status | Latex Agglutination Test | Serum Neutralization Test |
|---|---|---|---|---|---|---|
| Vaccinated | | | | | | |
| Matrouh | 45 | January 2, 2021 | June 2021 | Vaccinated | 42 | 45 |
| Halayeb and Shalaten | 36 | March 20, 2022 | November 2022 | Vaccinated | 33 | 36 |
| Total | 81 | | | | 75 | 81 |
| Non-Vaccinated | | | | | | |
| Matrouh | 40 | N/A | N/A | Non-vaccinated | 40 | 40 |
| Halayeb and Shalaten | 27 | N/A | N/A | Non-vaccinated | 27 | 27 |
| Menya | 15 | Non Vaccinated | 15 | 15 | Menya | 15 |
| Total | 82 | | | | 82 | 82 |

Diagnostic Specificity: 100% Diagnostic Sensitivity: 92.59%

**TABLE 4. Statistical Analysis of Latex Agglutination Test: Analytical Specificity and Sensitivity Based on 163 Serum Samples from Different Regions in Egypt and Various Sheep and Goat Species.**

| **Sensitivity** | **92.593%** | **84.571% to 97.233%** |
|---|---|---|
| Specificity | 100.000% | 95.601% to 100.000% |
| Area under curve | 0.963 | 0.921 to 0.986 |
| Positive predictive value | 100.000% | 95.200% to 100.000% |
| Negative predictive value | 93.182% | 86.354% to 96.723% |
| Accuracy | 96.319% | 92.161% to 98.637% |

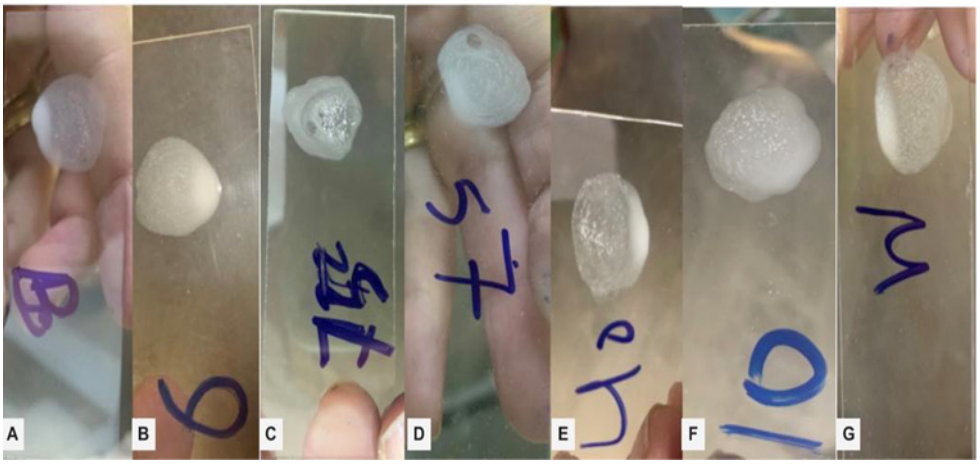


**Fig. 2. Shows the outcomes of a separate serum sample from sheep and goats injected with a local inactivated RVF vaccine and tested using the Latex Agglutination test.**

**Fig. 2: The results of serum samples obtained from sheep and goats injected with a local inactivated RVF vaccine and tested using the latex Agglutination test. Equal amount (10µl) of serum sample and inactivated virus coated on to the latex beads was put on clean glass slide and mixed together using a clean toothpick and observed for clump formation indicative of agglutination within one minute. Results of the test classified as weak positive (A and B), positive (C and D), and strong positive (E and F). G served as controlpositive.**

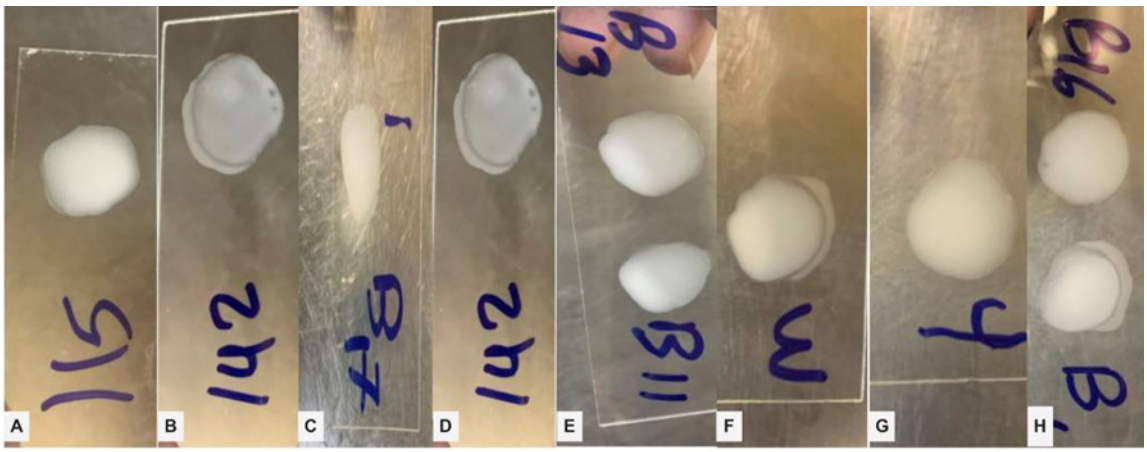


**Fig. 3. Illustrate the results of random serum samples from non -vaccinated sheep and goats as evaluated by latex agglutination test :**

**Fig. 3: Evaluation of Latex Agglutination Test for Random Serum Samples from Non-Vaccinated Sheep and Goats. To perform the test, equal amounts (10µl) of serum sample and inactivated virus coated on latex beads were mixed together on a clean glass slide using a toothpick. The absence of agglutination or clump formation (A-G) indicates a negative result for latex agglutination test. A control negative sample (F) was included for comparison.**

**TABLE 5. Sensitivity and Specificity of LAT compared to SNT for RVF Diagnosis for 163 sheep and goats serum sample**

| Test Method | True Positive | False positive | True negative | False-negative | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|
| LAT Assay | 75 | 0 | 82 | 6 | 92.5 | 100 |

*Ethical of approval*

All experimental animal procedures were conducted in compliance with the protocols approved by the AEC, under the application number (vet CU 08072023675), and sponsored by the Faculty of Veterinary Medicine, Cairo University.

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# تقنية تراص اللاتكس الجديدة للكشف السريع عن الاجسام المضادة لحمي الوادي المتصدع

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**المستخلص**

حمي الوادي المتصدع مرض مشترك بين الانسان والحيوان وله مخاطر جوهرية علي الصحة العامة في بعض مناطق افريقيا وشبة الجزيرة العربية .فهو يصيب كل من الانسان والمجترات .التشخيص السريع والدقيق اثناء تفشي المرض ضروري لتنفيذ خطط السيطرة الناجحة .هذه الدراسة تركز علي تطوير وتقييم طريقه تشخيصية جديدة والتي تستغل اختبار تراص اللاتكس للكشف عن الاجسام المضادة لفيروس حمي الوادي المتصدع .النتائج اظهرت دقة تشخيصية عالية حوالي% 91.2 .هذا الاختبار يحقق حساسية تشخيصه حوالي 92.59% ف التعرف علي عينات السيروم الايجابية وكذلك خصوصية تشخيصية حوالي100%..هذا الاختبار بسيط وسريع ورخيص الثمن وله امكانات كبيرة في فحص الحيوانات المصابة بحمي الوادي المتصدع علي مجال واسع .الخطوط العريضة لهذه الدراسة ان هذا الاختبار له تداعيات كبيرة نحو السيطرة علي انتشار مرض حمي الوادي المتصدع وتقليل تأثيره علي صحة الحيوانات والانسان.

**الكلمات الدالة**: حمي الوادي المتصدع ، امراض مشتركة ، الكشف عن الفيروس ، تراص اللاتكس ، اعتبارات الصحة العامة ، تقنية التشخيص.