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Compatibility of Lignocaine with Equine Rabies Immunoglobulin: An In Vitro Study Supporting Pain Management During Rabies Post-Exposure Prophylaxis

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Abstract

Rabies Immunoglobulin (RIG) infiltration in and around the wound is necessary as part of post-exposure prophylaxis (PEP) in patients with Category III exposure to potentially rabid animals. However, pain from RIG infiltration remains a significant challenge. This unnecessary iatrogenic pain can be avoided/ reduced by the topical application of local anaesthetics like lignocaine, using a lignocaine medicated pad at the site, 20 minutes before administration of RIG. We have earlier demonstrated its effectiveness through a clinical trial. However, concerns about a possible interaction between lignocaine and RIG must be addressed before translation of the practice into policy. This study evaluated the potential impact of lignocaine on the virus-neutralizing activity of equine RIG *in vitro*.

Baby Hamster Kidney (BHK21) cells were first exposed to lignocaine to assess cytotoxicity, with viable cell counts determined using trypan blue exclusion. Modified Rapid Fluorescent Focus Inhibition Test (RFFIT) was then used to compare rabies virus-neutralizing antibody (RVNA) titres of lignocaine-supplemented and untreated RIG. The study found that BHK21 cells remained viable at lignocaine concentrations up to 1/16 dilution, with no cytotoxicity observed at this level. Importantly, RVNA titres showed no significant differences between lignocaine-treated and untreated RIG preparations, demonstrating equivalent virus-neutralizing efficacy.

These findings support the use of lignocaine as a local anaesthetic during RIG administration without compromising therapeutic effectiveness. It offers a practical solution to enhance patient comfort during rabies PEP, especially in resource-limited settings. This study underscores the potential for integrating lignocaine into RIG administration protocols to provide compassionate and effective rabies prophylaxis.

Introduction

Rabies is a viral encephalitis caused by an RNA virus (Genus: *Lyssavirus*, Family: *Rhabdoviridae*) (Rupprecht 1996) and is practically 100% fatal even today (Baxter 2012). India reports about 36% of the total global deaths due to human rabies(Baxter 2012). Animal exposures are categorised based on WHO guidelines for initiation of post-exposure prophylaxis (World Health Organisation 2023). Single or multiple transdermal bites or scratches, licks on broken skin, and contamination of mucous membrane with saliva (i.e. licks) are considered Category III severe exposures and require administration of Rabies immunoglobulin (hyperimmune serum) and anti-rabies vaccine (World Health Organisation 2023). RIG is also indicated in Category II exposures in immunocompromised individuals. A significant challenge for both healthcare providers and patients is the iatrogenic pain caused by RIG infiltration into and around the wound (Haradanhalli et al. 2022). Addressing this pain is crucial to improving the patient experience during rabies PEP.(Haradanhalli et al. 2022)

Lignocaine (1–4%) has been proven to be useful for pain relief during suturing of lacerated wounds in children (Lambert and Goldman 2018) for topical anesthesia and skin-harvest sites (Jellish et al. 1999). Lignocaine binds to the sodium channels on the internal surface of the nerve cell membrane and locks them in the open state, thus preventing depolarisation (Beecham et al. 2025). It provides local and

topical anesthesia with an onset of action 3 to 5 minutes after application (Beecham et al. 2025). We have conducted a clinical trial previously, which showed that the application of 2% Lignocaine Medicated Pad (LMG) effectively reduces pain during RIG (Rabies antiserum IP - Equine) administration at the local site (Nujum et al. 2021). Translating this research finding into policy and practice will require understanding the interactions of lignocaine with rabies immunoglobulin at the tissue level.

Drug interactions between various medications have been documented to augment the efficacy of treatment with some acting synergistically, while others antagonistically (Boobis et al. 2009). Local anesthetics like procaine have been found to decrease the efficacy of drugs like amino salicylic acid and sulfonamides (WebMD 2023). So a concern of the potential interaction between Lignocaine and Equine Rabies Immunoglobulin could limit its use among practitioners.

Hence, it is imperative to prove that there is no reduction of the neutralisation of the virus by immunoglobulin due to the local application of lignocaine. This *in vitro* study was therefore carried out to assess the potential impact of 2% lignocaine on the virus-neutralising efficacy of rabies immunoglobulin (Rabies Antiserum IP - Equine) in cell culture.

Materials and Methods

The study was designed to evaluate the effect of lignocaine-supplemented hyperimmune serum that may impact performance of RFFIT and RVNA titres when compared to hyperimmune serum and standard rabies immunoglobulin. The study was also aimed to demonstrate the effect of lignocaine on BHK21 cell lines and virus infectivity in BHK21 cells. It was done at the State Institute of Animal Diseases, Palode, Kerala and did not require approval from ethics committee.

Commercial preparation of lignocaine, Loxicard with 2% lignocaine was selected for the study as it is devoid of preservatives other than sodium chloride. Two commercially available preparations of Rabies antiserum IP - Equine (300 IU/ml) viz., Equirab and VINS were procured from the pharmacy. Baby Hamster Kidney (BHK2) cell line [ATCC Cat. No. CCL-10] and Challenge Virus Standard (CVS-11 rabies virus strain) were obtained from NIMHANS, Bengaluru. Iscove's Modified Dulbecco's medium (IMDM), Cat. No. 17633 and Foetal Calf Serum (FCS), Cat. No. F7524 was procured from Sigma-Aldrich. Rabies FITC conjugate was purchased from Biorad (Cat. No. 3572112)

Determining the cytotoxicity of lignocaine

An initial investigation was conducted using Loxicard (Lignocaine 2%) to determine the cytotoxicity of the local anesthetic. Baby Hamster Kidney (BHK21) cells were treated against two-fold dilutions of Loxicard in a 96-well cell culture plate containing growth media Iscove's Modified Dulbeco's medium (IMDM) and incubated at 37°C for 24 hours. The incubated plates were observed for cell growth.

Relative potency and safety of lignocaine on BHK21 cells were determined using direct microscopy and trypan blue exclusion assay. Trypan blue exclusion assay was used to determine the percentage of

viable cells having clear cytoplasm and dead cells (blue cytoplasm) after exposure to lignocaine. Briefly, a mixture of 10ul of 0.4% trypan blue and 10ul of cell suspension was incubated at room temperature for three minutes. The mixture was applied to a hemocytometer and quantified under an inverted microscope.

Modified Rapid Fluorescent Focus Inhibition Test (RFFIT)

The RFFIT is presently the gold standard test used to monitor immune response to vaccination against rabies and for the quality testing of rabies hyperimmune serum (Lyng 1994). RFFIT assay performed here is adapted to 96-well tissue culture plates, which were followed in WHO-approved testing centres. The cell-safe concentration of lignocaine was added to two-fold dilutions (1/32 – 1/4096) of Rabies antiserum IP - Equine (300 IU/ml) viz., Equirab and VINS at 7.5 IU/ml and 15 IU/ml in a 96-well cell culture plate and incubated for one hour. Also, commercially available Equine Rabies Immunoglobulin preparation, Equirab calibrated against international reference serum having the potency of 30 IU/ml was used as reference anti-rabies serum (standard rabies immunoglobulin). Challenge virus standard [CVS-11 rabies virus strain] at 100FFD50 concentration was added to the hyperimmune serum lignocaine mixture in each well and the plates were again incubated for one hour. BHK 21 cells with a seeding density of 10/well were added. Reference standards, virus control, and cell control were also maintained. All tests were conducted in triplicate. RVNA neutralises the rabies virus and prevents infection of baby hamster kidney (BHK-21) cells susceptible to the rabies virus. Live virus infecting BHK 21 cells at higher serum dilutions was detected using Fluorescein isothiocyanate (FITC) conjugated Ab to the rabies virus. The serum neutralisation endpoint titre (ED50) is defined as the reciprocal dilution of the highest serum dilution at which 50% reduction in the number of fluorescent positive fields is observed as compared to the virus control. This RVNA titre (50% neutralization, ED50) is converted into IU/ml by calibrating against the reference standard.

Results

Lignocaine-mediated cell cytotoxicity was observed up to 1/16th dilution of local anesthetic (2% Loxicard). BHK21 cells were observed not adherent to the surface of the 96 well plates in neat and ½ dilution of Loxicard and cell attachment was disrupted upto ⅛th dilution. Wells at 1/16th dilution of Loxicard showed normal cell growth and monolayer was found and observed after 24 hours of incubation (Fig. 1). After that, Trypan exclusion studies also showed a similar pattern of cell growth on application of lignocaine. 100% cell death was observed in neat and ½ dilution of lignocaine. From ¼th dilution, the percentage of live cells increased from 20% to 60% at ⅛ th dilution and 1/16th dilution onwards 100% viable cell count was obtained. 1/16th dilution of Loxicard was utilised for modified RFFIT studies. The modified test was validated using 2 IU/ml standard rabies immunoglobulin. The available market preparations of hyperimmune serum (300 IU/ml) were diluted to 3.75 IU/ml, 7.5 IU/ml, and 15 IU/ml in IMDM as higher concentrations of RVNA can impact the traditional RFFIT assay.

No difference in RVNA titre was observed between lignocaine-supplemented hyperimmune serum and local anesthetic-devoid serum. (Table 1.) Fluorescent foci were started from 1/512th dilution (3.75IU/ml), 1/1024 dilution (7.5 IU/ml), and 1/2048 dilution (15 IU/ml), (Fig. 2.). Similar results were obtained in untreated hyperimmune serum. We could demonstrate similar RVNA titers by repeating the RFFIT assay with different lots of hyperimmune serum using the same test parameters. All virus controls showed 80–100% fluorescent foci, all cell controls remained negative in every assay and standard RIG showed fluorescent foci starting at 1/256th (2 IU/ml).

Table 1
Rabies Virus Neutralising Antibody titres in untreated and Lignocaine-treated ERIG

Reciprocal of Dilution	**RVNA Titre Value(IU/ml) Untreated ERIG (2IU/ml)**	**RVNA Titre Value(IU/ml) Lignocaine Treated ERIG (2IU/ml)**
128	0.94	0.94
256	1.87	1.87
512	3.75	3.75
1024	7.50	7.50
2048	15.0	15.0

Discussion

We have previously demonstrated that lignocaine (2%) impregnated cotton pads can effectively reduce pain during RIG administration (Nujum et al. 2021). However, before recommending this practice for policy implementation, we sought to investigate the potential interaction between lignocaine and RIG. The primary objective of this study was to determine whether lignocaine, when used as a local anaesthetic to reduce pain, affects the neutralising antibody activity of RIG. Since an in vivo assessment of this interaction was not feasible, we conducted an *in vitro* experiment to evaluate the potential impact.

Our study demonstrated that lignocaine does not compromise the efficacy or viral neutralisation process of RIG in infected BHK21 cells, as evidenced by unchanged RVNA titers in both control and lignocaine-treated samples.

Elective surgeries and anaesthesia are known risk factors for rabies vaccination failure. In emergencies, the potential immunomodulatory effects of anaesthesia and surgery should be considered, as they may hinder ARV efficacy (12,13). Moreover, avoiding local anaesthetics for postoperative analgesia is recommended to aid early detection of neurological deficits (Rewari et al. 2010).

Although some studies have explored interactions between local anaesthetics and immunoglobulins in general (Hijlstra et al. 1980), there is limited research specifically addressing the concurrent use of local

anaesthetics with immunoglobulin therapies. To our knowledge, this is the first *in vitro* study evaluating the interaction between a local anaesthetic and rabies immunoglobulin.

There may be concerns that local lignocaine application could dilute RIG. However, studies show that diluting RIG with solvents like normal saline up to 16-fold does not affect its efficacy (Madhusudana et al. 2013). RIG is routinely diluted two- to threefold as per national guidelines, especially in children where smaller doses must cover multiple/larger wound areas (ScienceDirect 2023). Thus, dilution from lignocaine is unlikely to be problematic.

Moreover, lignocaine is applied locally with minimal systemic absorption. Lidocaine patches, designed for targeted application, deliver only about 3 ± 2% of the applied dose into the bloodstream, with peak plasma concentrations remaining below 0.3 µg/mL — far below levels associated with systemic effects or toxicity (18,19).

Lignocaine (2%) impregnated cotton pads have proven effective in significantly reducing pain during RIG infiltration (Nujum et al. 2021). This laboratory evidence supports translating these findings into policy. The maximum lignocaine quantity used in the study was 5 mL, making it a highly affordable solution compared to commercial lignocaine patches, which range from Rs. 100 to Rs. 3599 (IndiaMART 2023a). Two percent lignocaine is widely available in 30 mL ampoules for just Rs. 20 to Rs. 30 (IndiaMART 2023b), lignocaine-soaked pads provide a practical, cost-effective strategy to minimize iatrogenic pain during RIG administration, particularly in resource-limited settings. The potential for lignocaine patches to interfere with vaccine efficacy is an important concern which can be dismissed from the evidence that this study provides. Studies assessing antibody responses to DTaP-IPV-Hib and hepatitis B vaccines in patients who received lidocaine-prilocaine (EMLA) patches for pain relief found no adverse impact on immunogenicity (Halperin et al. 2002). These findings suggest that local anaesthetic application does not compromise vaccine efficacy in general. Nonetheless, further research is warranted to confirm this in the context of rabies post-exposure prophylaxis (PEP), particularly in patients receiving local anaesthetic patches or applications alongside RIG.

Conclusions and Recommendations

The absence of a significant difference in RVNA titres between lignocaine-supplemented and untreated hyperimmune serum confirms that lignocaine does not affect eRIG efficacy. This supports its safe use to reduce iatrogenic pain during RIG administration. While these results are encouraging, further studies are needed to assess whether local anaesthetics impact rabies vaccine responses, particularly in PEP scenarios. Local anaesthesia during RIG infiltration is a simple, cost-effective intervention that addresses an often-overlooked aspect of care; preventing unnecessary pain and suffering. This approach can improve patient comfort, ensuring rabies prophylaxis is both effective and compassionate.

Declarations

Author statements

ZTN, MRR and SSA conceptualized and designed the study; PPR, DG and AS were instrumental in getting doing the lab work, ZTN did the initial drafting of paper, MRR RK SSG critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. ZTN and PPR are guarantors of the paper.

Declaration by authors

The manuscript has been read and approved by all the authors. The requirements for authorship have been met. Each author believes that the manuscript represents honest work. All authors declare that they have no conflict of interest to disclose.

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References

1. Baxter JM (2012) One in a million, or one in thousand: What is the morbidity of rabies in India? J Glob Health 2:010303.
2. Becker DE, Reed KL (2012) Local anesthetics: review of pharmacological considerations. Anesth Prog 59:90–102.
3. Beecham GB, Nessel TA, Goyal A (2025) Lidocaine. In: StatPearls. StatPearls Publishing, Treasure Island.
4. Boobis A, Watelet JB, Whomsley R, Benedetti MS, Demoly P, Tipton K (2009) Drug interactions. Drug Metab Rev 41:486–527.
5. Fescharek R, Franke V, Samuel MR (1994) Do anaesthetics and surgical stress increase the risk of post-exposure rabies treatment failure? Vaccine 12:12–13.
6. Ghodki P, Katikar M, Kulkarni P (2021) Dog bite and antirabies vaccination: what the anaesthesiologist needs to know. Indian J Anaesth 65:632–633.
7. Halperin BA, Halperin SA, McGrath P, Smith B, Houston T (2002) Lidocaine-prilocaine patch and vaccine antibody response in infants. Pediatr Infect Dis J 21:399–405.
8. Haradanhalli RS, Fotedar N, Kumari N, Narayana DHA (2022) Safety and clinical efficacy of human rabies immunoglobulin in post exposure prophylaxis for category III animal exposures. Hum Vaccin

Immunother 18:2081024.
9. Hijlstra A, Van Dorp W, Daha MR, Leslie GQ (1980) Effect of lidocaine on processing of immune aggregates by macrophages. Immunology 41:237–244.
10. Jellish WS, Gamelli RL, Furry PA, McGill VL, Fluder EM (1999) Effect of topical local anesthetic application to skin harvest sites for pain management in burn patients undergoing skin grafting. Ann Surg 229:115–120.
11. Lambert C, Goldman RD (2018) Pain management for children needing laceration repair. Can Fam Physician 64:900–902.
12. Lyng J (1994) Calibration of a replacement preparation for the international standard for rabies immunoglobulin. Biologicals 22:249–255.
13. Madhusudana SN, Ashwin BY, Sudarshan S (2013) Feasibility of reducing rabies immunoglobulin dosage for passive immunization against rabies. Hum Vaccin Immunother 9:1914–1917.
14. Navez ML, Monella C, Bösl I, Sommer D, Delorme C (2015) 5% lidocaine medicated plaster for treatment of postherpetic neuralgia. Pain Ther 4:1–15.
15. Nujum ZT, Valamparampil MJ, Rajagopal MR, Rahul A, Siju NS, Prajitha KC (2021) Efficacy of 2% lignocaine medicated pad for pain alleviation during rabies immunoglobulin administration: a randomized controlled trial. Indian J Public Health 65:356–361.
16. Rewari V, Garg R, Trikha A (2010) Rabies vaccine and neuraxial anaesthesia: case study. S Afr J Anaesth Analg 16:32–34.
17. Rupprecht CE (1996) Rabies virus. In: Baron S (ed) Medical Microbiology, 4th edn. University of Texas Medical Branch, Galveston.
18. World Health Organization (2023) Rabies vaccinations and immunization. https://www.who.int.

Figures

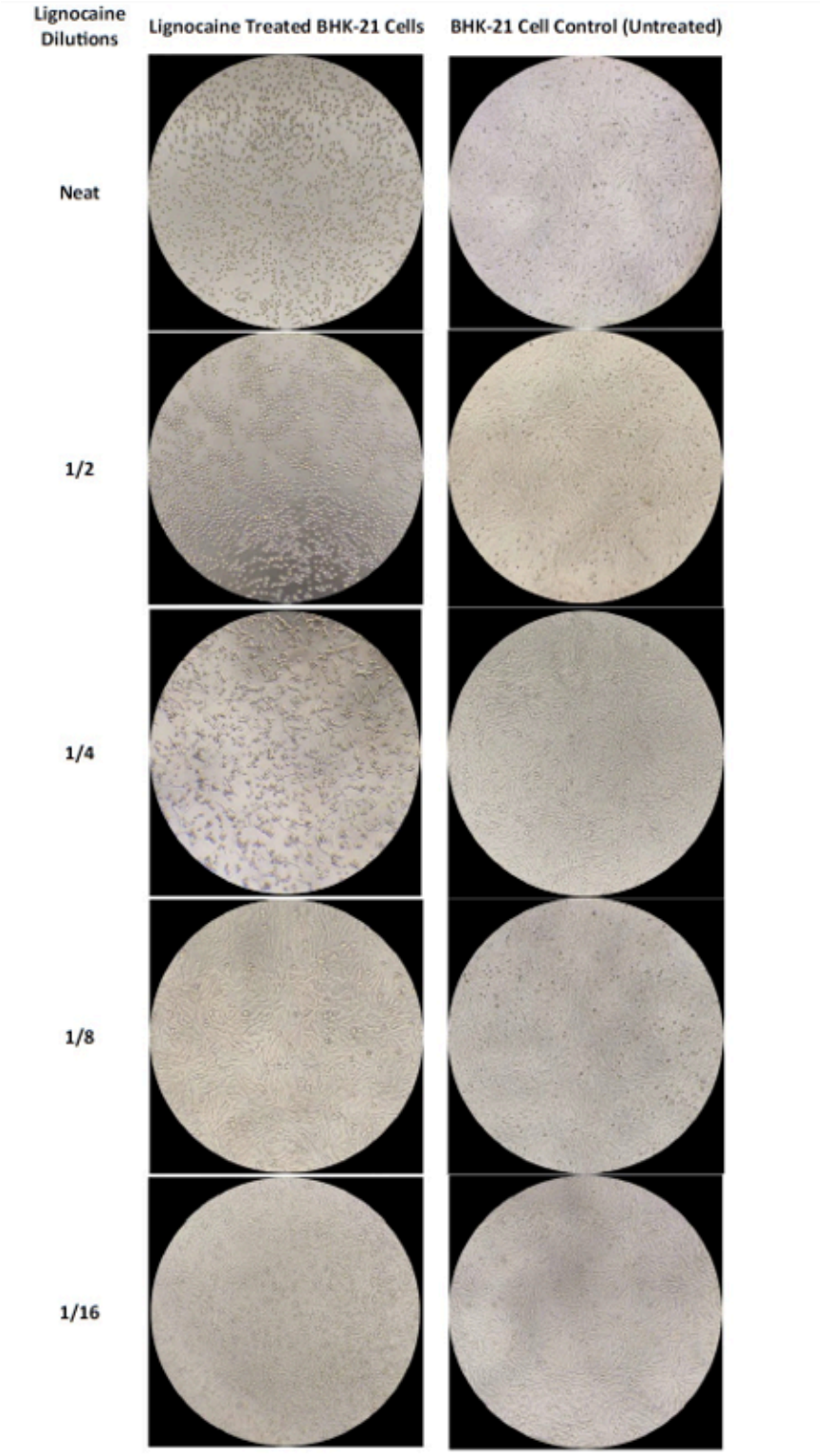


Figure 1

Lignocaine-Mediated Cytotoxicity in Lignocaine-treated and untreated control of BHK21 cells

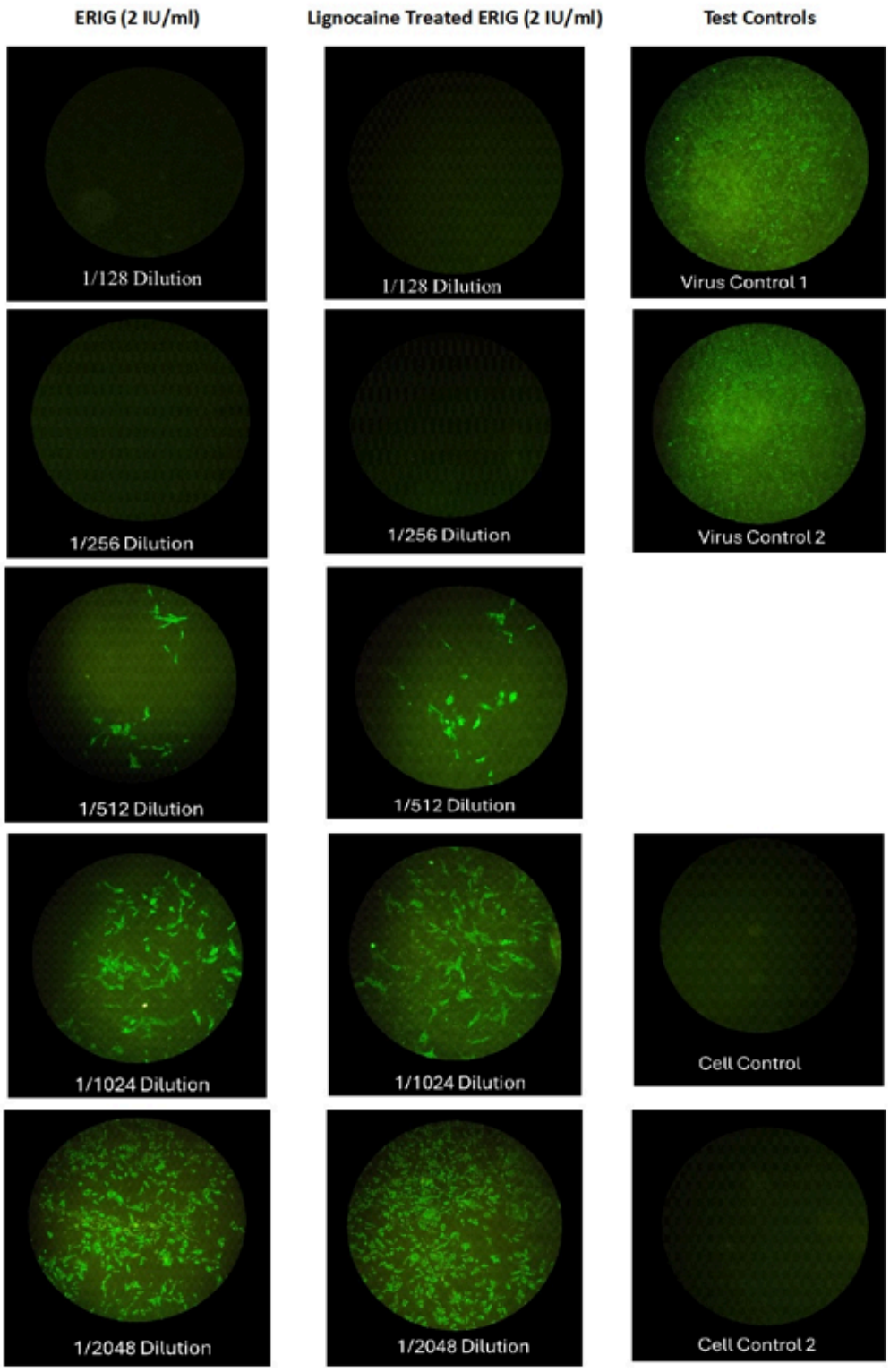


Figure 2

Rabies Virus Neutralisation shown by fluorescent foci in untreated and Lignocaine-treated ERIG