

# Differential profiles of mammalian and mosquito derived Ross River virus

Jade Redfern BForSc, BAppSc (Hons).

Faculty of Applied Science

University of Canberra,

Bruce, Canberra, ACT

Australia

A dissertation submitted as fulfilment of the requirements for the  
award of a Doctor of Philosophy in Applied Science.

April 2015

# Abstract

---

Ross River virus (RRV) is a mosquito borne virus that results in polyarthritic disease with symptoms including rash, lethargy and myalgia. Currently, RRV is responsible for around 5000 infections each year in Australia and disease symptoms can persist for several months or years in RRV infected patients. Early interaction of the virus with the host immune system has been identified as critical to the resulting disease. In addition, it has been shown that there are differences between mammalian and mosquito derived virus in the N-linked glycans on envelope proteins, the ability of the virus to infect cells *in vitro* and the resulting cytokine expression. However, the mechanism behind these differences and their effect *in vivo* is yet to be elucidated. The work reported in this thesis aimed to test the hypothesis that mammalian and mosquito derived RRV differ in replication fitness *in vitro* and *in vivo*, and are associated with differences in host response and the subsequent clinical disease.

RRV-T48, the common laboratory strain of RRV, showed differences in replication *in vitro*. Mammalian derived RRV T48 (Mam-RRV-T48) showed a replication advantage in mammalian (Vero) cells and a disadvantage in mosquito (C6/36) cells. In contrast, mosquito derived RRV T48 (Mos-RRV-T48) demonstrated a replication advantage in C6/36 cells and a disadvantage in Vero cells. This is indicative of RRVs ability to adapt to different host cells and increase the infectivity in the host within one passage. Furthermore, Mos-RRV-T48 showed a replication advantage in Raw 264.7 cells. Previous studies have also showed that mosquito derived virus had increased binding affinity

with cell surface receptors such as DC-SIGN, resulting in an increased infectivity. This is likely due to high mannose N-linked glycans present on mosquito derived virus only. Glycosylation differences have been found to result in strong IFN induction for mammalian but not mosquito derived virus in DCs. We confirmed this occurs in RRV-T48 infection of Raw 264.7 and Jaws II cells.

The RRV-T48 studies were expanded further utilising RRV field isolates extracted from mosquitos in Western Australia. The aim of this study was to examine if the differential effect observed with RRV-T48 could also be observed using wild type circulating RRV isolates *in vitro*. The study confirmed that RRV isolates mimicked the same pattern of infection as seen in RRV-T48 study. Mam-RRV-isolates produced the highest titres in Vero cells while Mos-RRV isolates produced the highest titres in C6/36 cells. Furthermore, approximately half of all Mam-RRV isolates replicated to higher titres than their Mos-RRV counterparts in Raw 264.7 cells; indicating that binding affinity is not the only factor involved in RRV replication.

Subsequent *in vivo* studies showed a significant difference between Mam-RRV-T48 and Mos-RRV-T48. The *in vivo* pilot study also revealed that disease progression was influenced by the gender of mice. While female mice showed almost no difference in disease when inoculated with Mam-RRV-T48 or Mos-RRV-T48, disease measures in male mice were significantly more severe. This has led to the development of a male only mouse model of RRV disease which provides more robust and reproducible results.

In the *in vivo* model, Mos-RRV-T48 infection induced less weight gain and higher clinical scores compared to Mam-RRV-T48 inoculated mice. Additionally, Mos-RRV-T48 infection resulted in more inflammatory infiltrates, greater tissue and bone destruction present in quadriceps and ankle samples compared to Mam-RRV-T48 inoculated mice. IFN $\beta$  induction was upregulated in Mam-RRV-T48 infected mice at peak disease. Isolates RRV-M and RRV-R showed similar disease patterns as seen with RRV-T48. This demonstrates that the differential induction of disease by Mam-RRV and Mos-RRV also occurs for wild type circulating isolates.

In addition, Barmah Forest and Dengue viruses also showed differential profiles between mammalian and mosquito derived viruses *in vitro*, similar to RRV-T48 findings. This suggests that the observations in RRV infection may be more widely applicable to other arboviruses and warrants investigation.

In conclusion, the cell culture findings in this thesis suggest that arboviruses should be cultured in mosquito cell lines, ensuring that the data generated is related to natural infection and host response. Furthermore, one of the most pivotal outcomes of this thesis is in the refinement of the current RRV mouse model which now takes into account gender dimorphism. The improvement of the RRV mouse model is an important step forward in more reproducible arbovirus research.

# Acknowledgements

---

I would like to thank Dr Suresh Mahalingham who started me on my doctoral journey and for making it possible for me to pursue my love of science. However, I would not have been able to complete this journey if it had not been for my primary supervisor Dr Reena Ghildyal. Her patience, encouragement, and support has made all the difference, and I thank her for her wisdom of knowing when I needed pushing and when I needed space. I would like to thank my other supervisor Dr Michelle Nelson who has stuck with me throughout the candidature and supplied hours of experimental and editorial advice.

I would also like to thank all of the general staff and students at the University of Canberra, you have all helped me at one point or another whether technically or emotionally and I am thankful for being surrounded by such wonderful people. Particular credit goes to Jacqui Richards for her hours of work in the animal house.

To Dijana Townsend, my weathered war veteran who undertook this journey with me. There are no words to express my gratitude to you for all the times you have picked me up and helped me move forward. We have cried together and laughed together and our friendship means more to me than you will ever know. You lent me your strength, Thank you. I would also like to thank Thomas Townsend<sup>1</sup>, for his amazing patience and assistance with my formatting faux pas. Also for his willingness to volunteer his time, technical

---

<sup>1</sup> All images, unless otherwise referenced, were created by Thomas Townsend under instruction from the candidate – Jade Redfern.

expertise, and graphic design genius which allowed my sanity to remain intact.

I would especially like to thank my mother, father, family and friends. I thank them for their understanding and patience through this process. For letting me off on all the visits that I missed and things I forgot over the past few years. I love you all. To my husband, Hamish Podger, for his encouragement and support. It is rare to find someone who will give you the freedom to follow your crazy dreams. Thank you my love. And lastly to my daughter, Saige Podger. You are my wings, my inspiration to be a better person every day and I hope I make you proud. I love you with all my heart and soul.

# Table of Contents

---

Abstract	iii
Acknowledgements .....	vii
Certificate of Authorship of Thesis .....	ix
Table of Contents .....	xi
List of Figures	xvii
List of Tables	xxi
List of Abbreviations .....	xxiii
<b>Chapter 1 Introduction .....</b>	<b>1</b>
<b>1.1. Arboviruses .....</b>	<b>1</b>
<b>1.2. Mosquito-borne viruses of medical importance .....</b>	<b>2</b>
1.2.1. Taxonomy .....	3
1.2.2. Togaviridae .....	4
1.2.3. Alphavirus Virion and genome .....	7
1.2.4. Alphavirus replication .....	7
1.2.5. RRV epidemiology and disease .....	14
1.2.6. Flaviviridae .....	17
1.2.7. Bunyaviridae .....	22
<b>1.3. Arbovirus immunobiology and pathogenesis .....</b>	<b>25</b>
1.3.1. Cells targeted by arboviruses in infection and disease .....	25
1.3.2. Attachment and entry of arboviruses .....	27
1.3.3. Early events in host response to arboviral infection .....	28
1.3.4. Cytokines .....	35
1.3.5. Type I interferons (IFN) and arbovirus infection .....	39
<b>1.4. Arboviruses derived from mammalian versus mosquito cells..</b>	<b>43</b>
1.4.1. Glycosylation differences between mammalian and mosquito derived virus .....	44
1.4.2. Entry/attachment differences between mammalian and mosquito derived virus .....	47
1.4.3. Differential immune responses to mammalian and mosquito derived virus .....	48

<b>1.5. Mouse model of RRV.....</b>	<b>49</b>
<b>1.6. Thesis objectives.....</b>	<b>51</b>
<b>Chapter 2      Materials and Methods.....</b>	<b>53</b>
<b>2.1. General reagents .....</b>	<b>53</b>
<b>2.2. Cell lines and virus strains .....</b>	<b>54</b>
2.2.1. Cell banking and culture .....	55
<b>2.3. Virus propagation and purification .....</b>	<b>57</b>
2.3.2. RRV propagation and purification.....	61
2.3.3. Barmah forest virus (BFV) .....	61
2.3.4. Dengue virus (DenV).....	61
2.3.5. Virus titration by plaque assay (RRV and BFV) .....	62
2.3.6. DenV titration by immuno-focus assay.....	62
2.3.7. Purification of RRV and BFV .....	64
2.3.8. Purification of DenV .....	64
<b>2.4. Virus kinetics studies.....</b>	<b>65</b>
2.4.1. Infection of adherent cells.....	65
2.4.2. Infection of non-adherent cells.....	66
<b>2.5. Mouse Studies.....</b>	<b>67</b>
2.5.1. Mouse strains and breeding.....	67
2.5.2. Mouse model of disease. ....	67
<b>2.6. Expression studies .....</b>	<b>69</b>
2.6.1. RNA extraction from cultured cells .....	69
2.6.2. RNA extraction from mouse tissue.....	69
2.6.3. Preparation of complementary DNA (cDNA).....	70
2.6.4. Real time PCR.....	71
<b>2.7. Statistical analysis .....</b>	<b>73</b>
<b>Chapter 3      Characterisation of T48 Ross River virus</b>	
<b>replication in tissue culture .....</b>	<b>75</b>
<b>3.1. Introduction .....</b>	<b>75</b>
<b>3.2. Results.....</b>	<b>77</b>
3.2.1. Plaque morphology of Mam-RRV-T48 and Mos-RRV-T48 .....	77
3.2.2. Multistep replication kinetics of Mam-RRV-T48 and Mos-RRV-T48 in Vero, C6/36, Raw 264.7 and Jaws II cells .....	78

3.2.3. Induction of IFN $\beta$ by Mam-RRV-T48 and Mos-RRV-T48 in Raw 264.7 and Jaws II cells .....	81
<b>3.3. Discussion .....</b>	<b>83</b>
3.3.1. Differential plaque morphology of mammalian and mosquito derived RRV .....	83
3.3.2. Differential replication kinetics of mammalian and mosquito derived RRV in Vero and C6/36 cells .....	84
3.3.3. Differential replication kinetics of mammalian and mosquito derived RRV in Raw 264.7 and Jaws II cells .....	85
3.3.4. Differential induction of IFN $\beta$ by mammalian and mosquito derived RRV in Raw 264.7 and Jaws II cells .....	87
<b>3.4. Conclusion .....</b>	<b>88</b>
<b>Chapter 4 Characterisation of Ross River virus field isolates in tissue culture .....</b>	<b>91</b>
<b>4.1. Introduction .....</b>	<b>91</b>
<b>4.2. Results .....</b>	<b>93</b>
4.2.1. Multistep replication kinetics of Mam-RRV and Mos-RRV field isolates in Vero cells .....	93
4.2.2. Mam-RRV and Mos-RRV field isolate trends in Vero cells .....	94
4.2.3. Multistep replication kinetics of Mam-RRV and Mos-RRV field isolates in a mosquito cell line .....	99
4.2.4. Mam-RRV and Mos-RRV field isolate trends in a mosquito cell line .....	100
4.2.5. Multistep replication kinetics of Mam-RRV and Mos-RRV field isolates in Raw 264.7 cells .....	105
4.2.6. Mam-RRV and Mos-RRV field isolate trends in Raw 264.7 cells .....	106
<b>4.3. Discussion .....</b>	<b>111</b>
4.3.1. Differential replication kinetics in Vero and C6/36 cells .....	111
4.3.2. Differential replication kinetics in Raw 264.7 cells .....	112
<b>4.4. Conclusion .....</b>	<b>114</b>
<b>Chapter 5 Characterisation of a Ross River virus infection mouse model</b>	<b>115</b>
<b>5.1. Introduction .....</b>	<b>115</b>
<b>5.2. Results .....</b>	<b>119</b>
5.2.1. Disease profile of IFNAR -/- mice in RRV infection .....	119

5.2.2. Inactivated RRV in a mouse model of disease .....	124
5.2.3. Mam-RRV-T48 and Mos-RRV-T48 in a mouse model of disease .....	125
5.2.4. Cytokine expression of active and inactive Mam-RRV-T48 or Mos-RRV-T48 in a mouse model of disease. ....	127
5.2.5. Comparison of gender in mouse model of RRV infection .....	136
5.2.6. Viral titres in mice inoculated with Mam-RRV-T48 or Mos-RRV-T48 in mice.....	140
5.2.7. Histology of mice infected with Mam-RRV-T48 or Mos-RRV-T48.....	144
5.2.8. Cytokine profiles of mice inoculated with Mam-RRV-T48 or Mos-RRV-T48.....	154
<b>5.3. Discussion .....</b>	<b>164</b>
5.3.1. IFN $\beta$ is critical for development of disease symptoms.....	164
5.3.2. UV inactivated virus does not cause arthritic disease symptoms in the RRV mouse model of disease. ....	166
5.3.3. Mosquito derived RRV causes a decrease in percentage weight gain and an increase in clinical score in the RRV mouse model of disease. ....	167
5.3.4. Cytokine profiles of RRV disease depend on viral cell origin and differ from inactivated virus. ....	167
5.3.5. Results gained from the RRV mouse model are sexually dimorphic. ....	169
5.3.6. Male mice show greater reproducible variation when inoculated with Mam-RRV or Mos-RRV.....	170
5.3.7. Viral titres are higher in quadriceps muscle and ankle joint of Mos-RRV-T48 inoculated mice at peak disease .....	170
5.3.8. Mice have different histology when inoculated with Mam-RRV or Mos-RRV .....	171
5.3.9. Mice have different cytokine profiles when inoculated with Mam-RRV or Mos-RRV .....	172
<b>5.4. Conclusion.....</b>	<b>173</b>
<b>Chapter 6 Characterisation of Ross River virus isolates in a mouse model of disease.....</b>	<b>175</b>
<b>6.1. Introduction .....</b>	<b>175</b>
<b>6.2. Results.....</b>	<b>177</b>

6.2.1. Weight gain in mice inoculated with Mam-RRV-T48 or Mos-RRV-T48 field isolates.....	177
6.2.2. Clinical scores of mice inoculated with Mam-RRV or Mos-RRV field isolates. ....	182
6.2.3. Disease progression in mice inoculated with Mam-RRV or Mos-RRV field isolates.....	186
6.2.4. Viral titres in mice inoculated with Mam-RRV or Mos-RRV field isolates .....	190
6.2.5. Quadriceps muscle histology of mice inoculated with Mam-RRV or Mos-RRV field isolates at 24 hours post infection .....	194
6.2.6. Ankle joint histology of mice inoculated with Mam-RRV or Mos-RRV field isolates at 24 hours post infection.....	198
6.2.7. Brain histology of mice inoculated with Mam-RRV and Mos-RRV field isolates at 24 hours post infection.....	202
6.2.8. Quadriceps muscle histology of mice inoculated with Mam-RRV and Mos-RRV field isolates at peak disease.....	204
6.2.9. Ankle joint histology of mice inoculated with Mam-RRV or Mos-RRV field isolates at peak disease .....	208
6.2.10. Brain histology of mice inoculated with Mam-RRV or Mos-RRV at peak disease .....	213
6.2.11. Cytokine profiles of mice inoculated with Mam-RRV and Mos-RRV field isolates.....	215
<b>6.3. Discussion .....</b>	<b>228</b>
6.3.1. Weight gain and clinical score trends in mice inoculated with mammalian and mosquito derived RRV isolates. ....	229
6.3.2. Investigation of differential viral titres in tissue of mice inoculated with RRV field isolates. ....	231
6.3.3. Differential tissue pathology in mice inoculated with RRV field isolates at 24 hours post infection .....	232
6.3.4. Differential tissue pathology in tissue of mice inoculated with RRV field isolates at peak disease post infection .....	234
6.3.5. Cytokine profiles in quadriceps muscle of mice inoculated with RRV field isolates.....	236
6.3.6. Cytokine profiles in ankle joints of mice inoculated with RRV field isolates .....	238
<b>6.4. Conclusion.....</b>	<b>239</b>
<b>Chapter 7 Mammalian and mosquito derived arbovirus phenotypes 241</b>	

<b>7.1. Introduction .....</b>	<b>241</b>
<b>7.2. Results.....</b>	<b>243</b>
7.2.1. Plaque morphology of Mam-BFV and Mos-BFV.....	243
7.2.2. Multistep replication kinetics of Mam-BFV and Mos-BFV in Raw 264.7 cells .....	243
7.2.3. Differential IFN $\beta$ induction by mammalian and mosquito derived Barmah Forest virus in Raw 264.7 cells .....	244
7.2.4. Plaque morphology of Mam-DenV and Mos-DenV .....	246
7.2.5. Multistep replication kinetics of Mam-DenV and Mos-DenV in Jaws II cells .....	246
7.2.6. Differential IFN $\beta$ induction by DenV in Jaws II cells .....	247
<b>7.3. Discussion .....</b>	<b>248</b>
7.3.1. Differential plaque phenotypes of arboviruses.....	249
7.3.2. Differential growth kinetics of arboviruses in immune cell lines .....	250
7.3.3. IFN $\beta$ induction by mammalian and mosquito derived arboviruses .....	251
<b>7.4. Conclusion.....</b>	<b>252</b>
<b>Chapter 8      Discussion .....</b>	<b>255</b>
<b>8.1. Arbovirus growth kinetics in tissue culture .....</b>	<b>255</b>
<b>8.2. Gender dimorphism in the RRV mouse model of disease.....</b>	<b>259</b>
<b>8.3. Mammalian and mosquito derived RRV results in differential         disease profiles in a mouse model of disease.....</b>	<b>261</b>
<b>8.4. Trends in infection and disease caused by RRV field isolates in         a male mouse model.....</b>	<b>262</b>
<b>8.5. Inflammatory responses to RRV in vivo .....</b>	<b>268</b>
<b>8.6. Conclusions and future directions .....</b>	<b>273</b>
Appendix 1 – Mammalian and mosquito derived Ross river virus field isolate multistep kinetics in Vero cells.....	275
Appendix 2 – Mammalian and mosquito derived Ross river virus field isolate multistep kinetics in C6/36 cells .....	279
Appendix 3 – Mammalian and mosquito derived Ross river virus field isolate multistep kinetics in Raw 264.7 cells .....	283
References .....	287

# List of Figures

---

Figure 1.1	Schematic representation of alphavirus virion and genome.....	6
Figure 1.2	Alphavirus replication cycle .....	9
Figure 1.3	Relationship between rainfall, mosquito density and RRV incidence in Brisbane.....	15
Figure 1.4	Schematic representation of flavivirus virion and genome. ....	19
Figure 1.5	Flavivirus replication cycle .....	20
Figure 1.6	Schematic representation of Bunyaviridae virion and genome. ....	24
Figure 1.7	Comparative overview of mammalian and mosquito N-glycan subtypes .....	46
Figure 3.1	Differential plaque morphology of Mam-RRV-T48 and Mos-RRV-T48 .....	78
Figure 3.2	Growth kinetics of mammalian and mosquito derived RRV in various cell lines .....	80
Figure 3.3	IFN $\beta$ induction by Mam-RRV-T48 and Mos-RRV-T48 .....	82
Figure 4.1	RRV field isolate multistep kinetics in mammalian cells .....	97
Figure 4.2	RRV field isolate growth kinetic trends in mammalian cells...	98
Figure 4.3	RRV field isolate multistep kinetics in mosquito cells .....	103
Figure 4.4	RRV field isolate growth kinetic trends in mosquito cells.....	104
Figure 4.5	RRV field isolate multistep kinetics in mammalian macrophage cells .....	109
Figure 4.6	RRV field isolate growth kinetic trends in mammalian macrophages.....	110
Figure 5.1	Type I interferon is important in development of clinical disease in mouse model of Ross river virus infection .....	123
Figure 5.2	Disease progression in mice inoculated with RRV .....	126
Figure 5.3	Inflammatory cytokine profiles differ between mammalian and mosquito derived RRV and also between active and inactive RRV in the quadriceps muscle. ....	131

Figure 5.4	Inflammatory cytokine profiles differ between mammalian and mosquito derived RRV and also between active and inactive RRV in the ankle joint.....	135
Figure 5.5	Male C57Bl/6 mice infected with mosquito derived T48 RRV lose more weight and have higher clinical scores than female C57Bl/6 mice. ....	138
Figure 5.6	Difference in disease and weights of male C57Bl/6 mice inoculated with T48 RRV is highly reproducible. ....	139
Figure 5.7	Blood and tissue virus titres. ....	143
Figure 5.8	Histology of quadriceps muscle in mice infected with RRV at 24 hours post infection. ....	145
Figure 5.9	Histology of quadriceps muscle in mice infected with RRV at peak disease. ....	146
Figure 5.10	Histology of ankle joint in mice infected with RRV at 24 hours post infection. ....	149
Figure 5.11	Histology of ankle joint in mice infected with RRV at peak disease.....	151
Figure 5.12	Histology of brain tissue in mice infected with RRV at 24 hours and peak disease. ....	153
Figure 5.13	Cytokine profiles in the quadriceps muscle of mice inoculated with mammalian and mosquito derived RRV.....	158
Figure 5.14	Cytokine profiles in the ankle joint of mice inoculated with mammalian and mosquito derived RRV.....	163
Figure 6.1	Weight gain of C57Bl/6J mice inoculated with Mam-RRV and Mos-RRV field isolates.....	181
Figure 6.2	Clinical score of C57Bl/6J mice inoculated with Mam-RRV and Mos-RRV isolates. ....	185
Figure 6.3	Disease profile trends for mice inoculated with Mam-RRV and Mos-RRV.....	189
Figure 6.4	Viral titres recovered from blood, quadriceps muscle, ankle joint and brain of mice inoculated with Mam-RRV and Mos-RRV field isolates. ....	193
Figure 6.5	Histology of quadriceps muscle in mice infected with RRV at 24 hours post infection .....	197

Figure 6.6	Histology of ankle joint of mice infected with RRV at 24 hours post infection .....	201
Figure 6.7	Histology of brain tissue in mice infected with RRV at 24 hours post infection .....	203
Figure 6.8	Histology of quadriceps muscle in mice infected with RRV at peak disease .....	207
Figure 6.9	Histology of ankle joint in mice infected with RRV at peak disease.....	212
Figure 6.10	Histology of brain tissue in mice infected with RRV at peak disease.....	214
Figure 6.11	Cytokine profiles in the quadriceps muscle of mice inoculated with mammalian and mosquito derived RRV field isolates...	220
Figure 6.12	Cytokine profiles in the ankle joint of mice inoculated with mammalian and mosquito derived RRV field isolates. ....	226
Figure 7.1	Differential plaque morphology of mammalian and mosquito derived Barmah Forest virus .....	243
Figure 7.2	Growth kinetics of mammalian and mosquito derived Barmah forest virus in Raw 264.7 cells. ....	244
Figure 7.3	IFN $\beta$ induction by mammalian and mosquito derived Barmah forest virus.....	245
Figure 7.4	Differential plaque morphology of mammalian and mosquito derived dengue virus.....	246
Figure 7.5	Growth kinetics of mammalian and mosquito derived dengue virus in various Jaws II cells .....	247
Figure 7.6	IFN $\beta$ induction of mammalian and mosquito derived dengue virus .....	248

# List of Tables

---

Table 1.1	General information on arboviruses .....	4
Table 1.2	Notifications of Ross River virus infection .....	7
Table 1.3	Mosquito-borne viruses in the Flaviviridae family that cause human disease .....	17
Table 1.4	Dengue disease grade classifications .....	21
Table 2.1	General reagents and their preparation. ....	53
Table 2.2	Growth media. ....	54
Table 2.3	Cell lines and their normal growth conditions. ....	55
Table 2.4	<i>In-vitro</i> transcription reaction mix .....	59
Table 2.5	RRV field isolates .....	60
Table 2.6	Clinical score of RRV disease in mice .....	68
Table 2.7	Reverse transcriptase master mix reagent.....	70
Table 2.8	Real-time PCR master mix reagent.....	71
Table 2.9	Real time PCR parameters .....	72
Table 8.1	Correlation between <i>in vivo</i> and <i>in vitro</i> profiles of RRV isolates .....	263

# List of Abbreviations

---

<b>ADE</b>	Antibody-dependant enhancement
<b>ATF</b>	Activation Transcription Factor
<b>BFV</b>	Barmah Forest Virus
<b>BHK</b>	Baby Hamster Kidney Cells
<b>CP</b>	Capsid Protein
<b>C3</b>	Complement Component 3
<b>CDC</b>	Center for Disease Control
<b>cDNA</b>	Complementary DNA
<b>ChikV</b>	Chikungunya Virus
<b>CR3</b>	Complement Receptor 3
<b>DC</b>	Dendritic Cells
<b>DC-SIGN</b>	Dendritic Cell Specific Intercellular Adhesion Molecule-3-Grabbing Non-Integrin
<b>DenV</b>	Dengue Virus
<b>DF</b>	Dengue Fever
<b>DHF</b>	Dengue Haemorrhagic Fever
<b>DSS</b>	Dengue Shock Syndrome
<b>E</b>	Envelope Protein
<b>EEEV</b>	Eastern Equine Encephalitis Virus
<b>GAS</b>	INF $\gamma$ Activated Site
<b>GM-CSF</b>	Granulocyte Macrophage Colony Stimulating Factor
<b>HMG</b>	High Mobility Group Protein
<b>HS</b>	Heparin Sulfate
<b>IFN</b>	Interferon
<b>IFNAR</b>	Interferon Alpha Receptor
<b>IKK</b>	Inhibitor of Kappa Beta Kinase
<b>IL</b>	Interleukin
<b>IP</b>	Interferon $\gamma$ Induced Protein

<b>IRF</b>	Interferon Regulator Factor
<b>ISGF</b>	IFN-Stimulated Gene Factor
<b>ISRE</b>	IFN-Stimulated Response Element
<b>Jak-STAT</b>	Janus Kinase – Signal Transducer and Activator of Transcription
<b>JEV</b>	Japanese Encephalitis
<b>kb</b>	Kilobases
<b>KV</b>	Kunjin Virus
<b>LPS</b>	Lipopolysaccharide
<b>L-SIGN</b>	Liver/Lymph Node Intercellular Adhesion Molecule-3-Grabbing Non-Integrin
<b>Mam</b>	Mammalian Derived
<b>MBL</b>	Mannose Binding Lectin
<b>MCP/CCL2</b>	Monocyte Chemotactic Protein
<b>MHC</b>	Major Histocompatibility Complex
<b>MIF</b>	Macrophage Inhibitory Factor
<b>MIG</b>	Macrophage Induced Gene
<b>MIP</b>	Macrophage Inhibitor Complex
<b>Mos</b>	Mosquito Cell Derived
<b>mRNA</b>	Messenger RNA
<b>MVE</b>	Murray Valley Encephalitis
<b>NF-KB</b>	Nuclear Factor Kappa B
<b>NK</b>	Natural Killer cells
<b>NO</b>	Nitric Oxide
<b>NsP</b>	Non-Structural Protein
<b>ORF</b>	Open Reading Frame
<b>PCR</b>	Polymerase Chain Reaction
<b>PDC</b>	Plasmacytoid Dendritic Cells
<b>PKR</b>	Serine Threonine Protein Kinase R
<b>Ra</b>	Receptor Antagonist
<b>RdRp</b>	RNA-dependant RNA Polymerase

<b>RRV-T48</b>	Ross River Virus T48
<b>RVFV</b>	Rift Valley Fever
<b>SFV</b>	Semliki Forest Virus
<b>SinV</b>	Sinbis Virus
<b>SIRS</b>	Systemic Inflammatory Response Syndrome
<b>TBK</b>	Threonine Protein Kinase
<b>TC</b>	T Lymphocyte Cytotoxic Cells CD8+
<b>TGF</b>	Transforming Growth Factor
<b>TH</b>	T Lymphocyte Helper Cells CD4+
<b>TLR</b>	Toll Like Receptor
<b>TM</b>	T Lymphocyte Memory Cell
<b>TNF</b>	Tumour Necrosis Factor
<b>VEEV</b>	Venezuelan equine encephalitis virus
<b>WHO</b>	World Health Organisation
<b>WNV</b>	West Nile Virus
<b>Wt</b>	Wild Type
<b>YF</b>	Yellow Fever