

Outline of an innovative cross-species assay to evaluate *Lassa mammarenavirus* (Lassa fever) serology

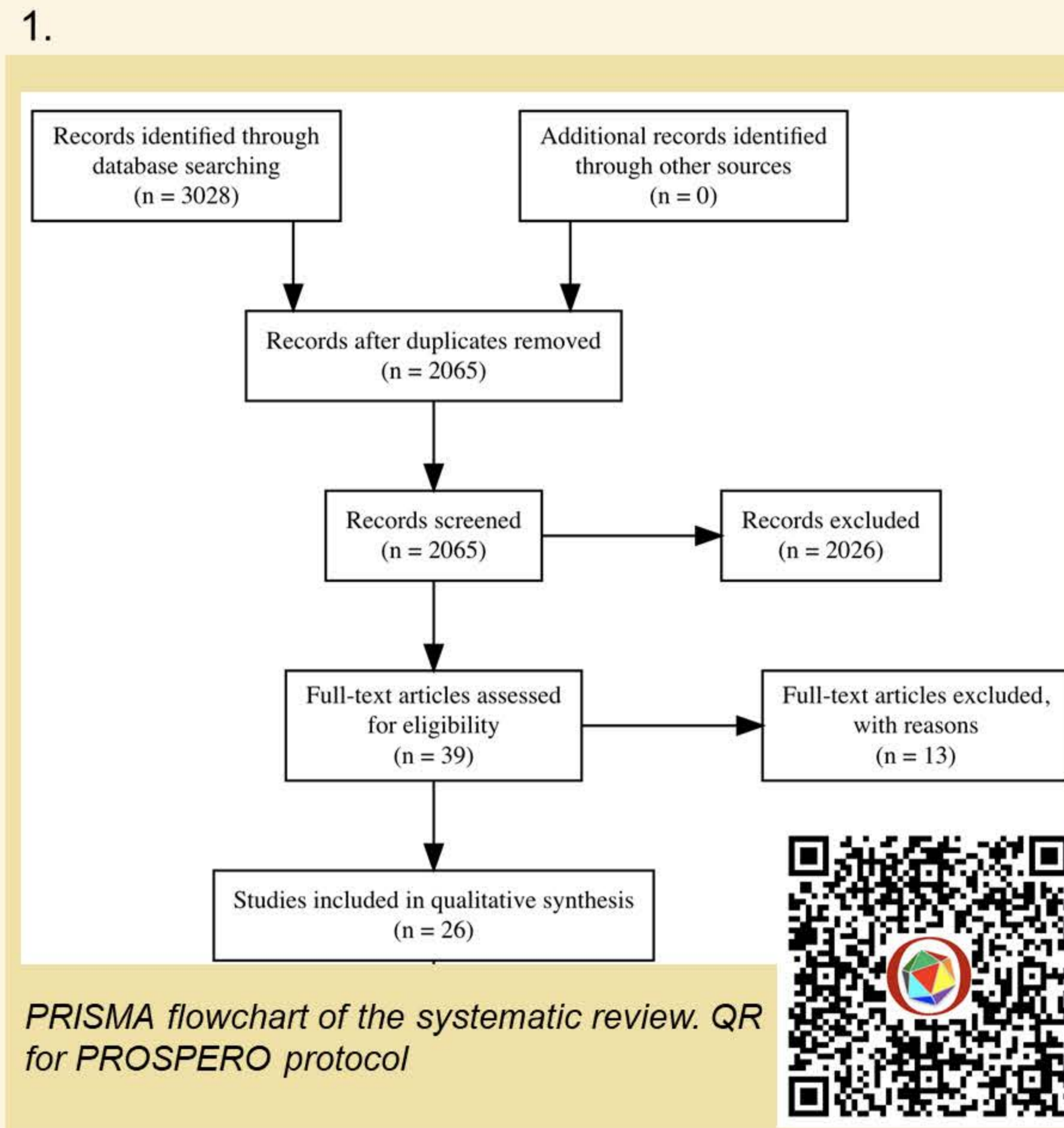
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Introduction

Lassa fever (LASV) is a zoonotic infectious disease endemic to West Africa that spills over to humans from the rodent species *Mastomys natalensis*. Additional rodent species in endemic regions demonstrate seropositivity for LASV but their role in its transmission remains unclear. The wider distribution of LASV in peri-domestic animals is unknown due to lack of suitable assays.

Current diagnostic assays

A systematic review of Lassa fever assays in non-humans was conducted to identify assays feasible for use in peri-domestic animals (Panel 1). The assays and their suitability are shown in Panel 2.



2.

Assay	Stage of infection	Cross-species	Infrastructural requirement	Lab requirements	Number of articles	N of animal orders*, across all papers	N of samples, across all papers
Viral Culture	Acute infection only	Species independent	High	Live virus (BSL4)	6	3	5459
IFA	Acute infection and seropositivity	Species dependent	Medium	BSL3	5	3	9147
ELISA antigen	Acute infection	Species independent	Medium	BSL3	1	1	1616
antibody ELISA	Seropositivity	Species dependent	Medium	BSL2	5	2	2507
RT-PCR	Acute infection	Species independent	High	BSL3	7	2	6384

No suitable assays were identified in the literature review.

Current species independent assays are limited to detecting acute infection. Those that can detect seroprevalence were identified to be effective in rodent and primate species only.

*Orders included: Primates, Rodents and Bats.

Luciferase ImmunoPrecipitation System (LIPS)

No assays were suitable for cross-species seroprevalence studies. The LIPS assay has been used for other diseases that affect multiple animal species and would be viable for our planned research.

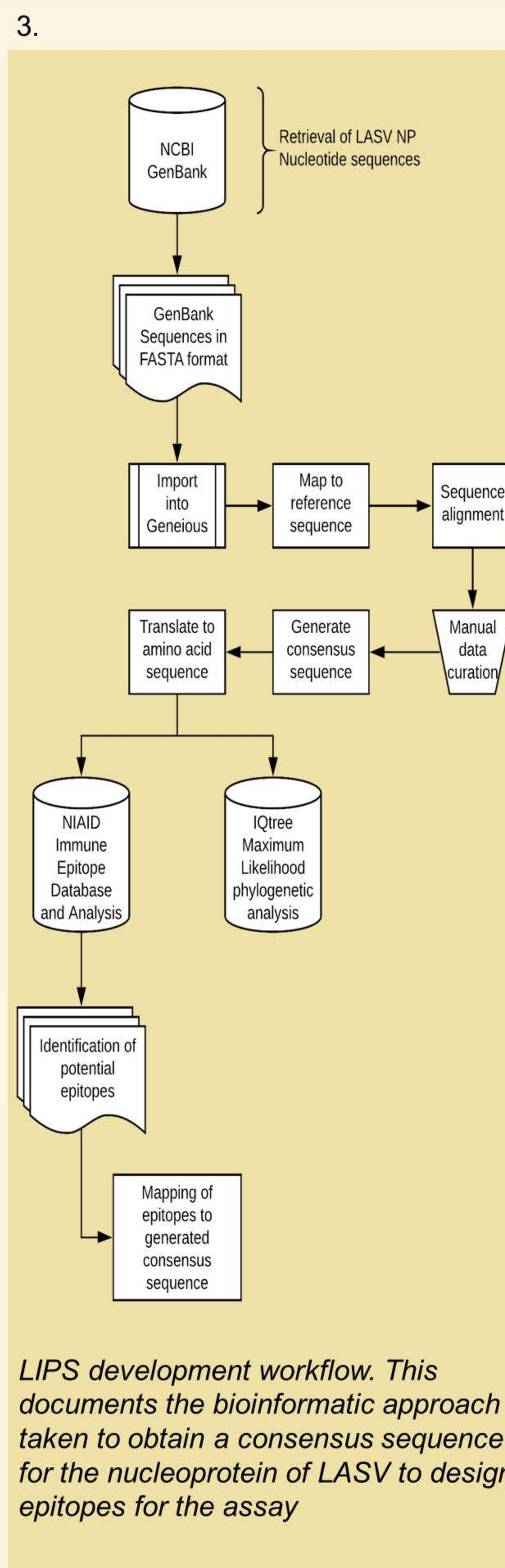
Our review identified that the nucleoprotein (NP) component of the virus was predominantly targeted by these assays. We focused our search for a suitable epitope on this region of the genome.

Obtaining NP sequences

676 complete sequences were obtained from the NCBI GenBank (Panel 4). The sequences were aligned in Geneious using ClustalW. A consensus sequences was constructed. The workflow is demonstrated in Panel 3.

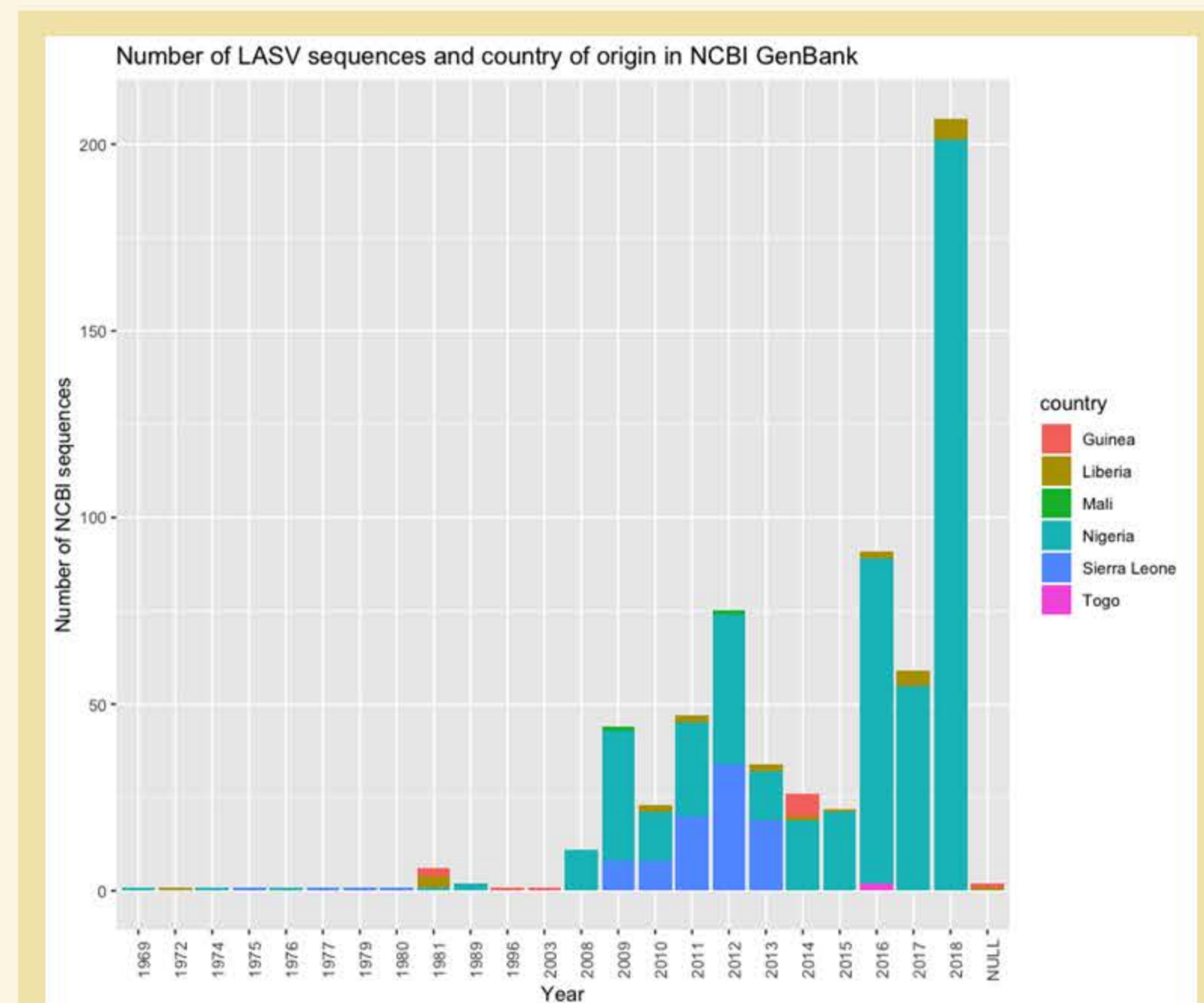
Antigen screening

Potential antigens within the consensus sequence were identified using the NIAID immune epitope database and analysis resource. Bepipred, Bepipred-2 and Kolaskar-Tongoankar algorithms were used to score the antigenicity of different regions of the sequence. The analysis is presented in Panel 6.



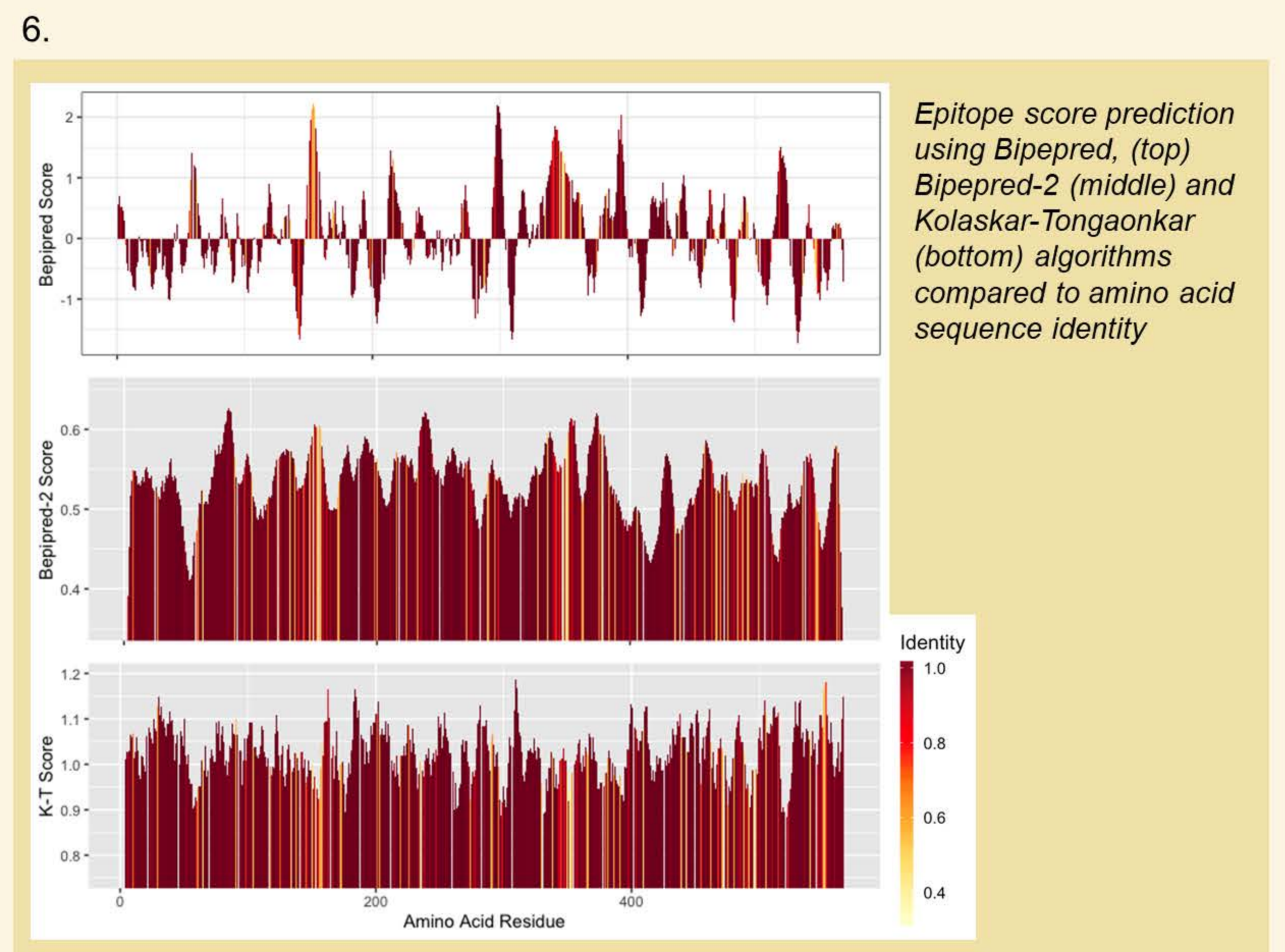
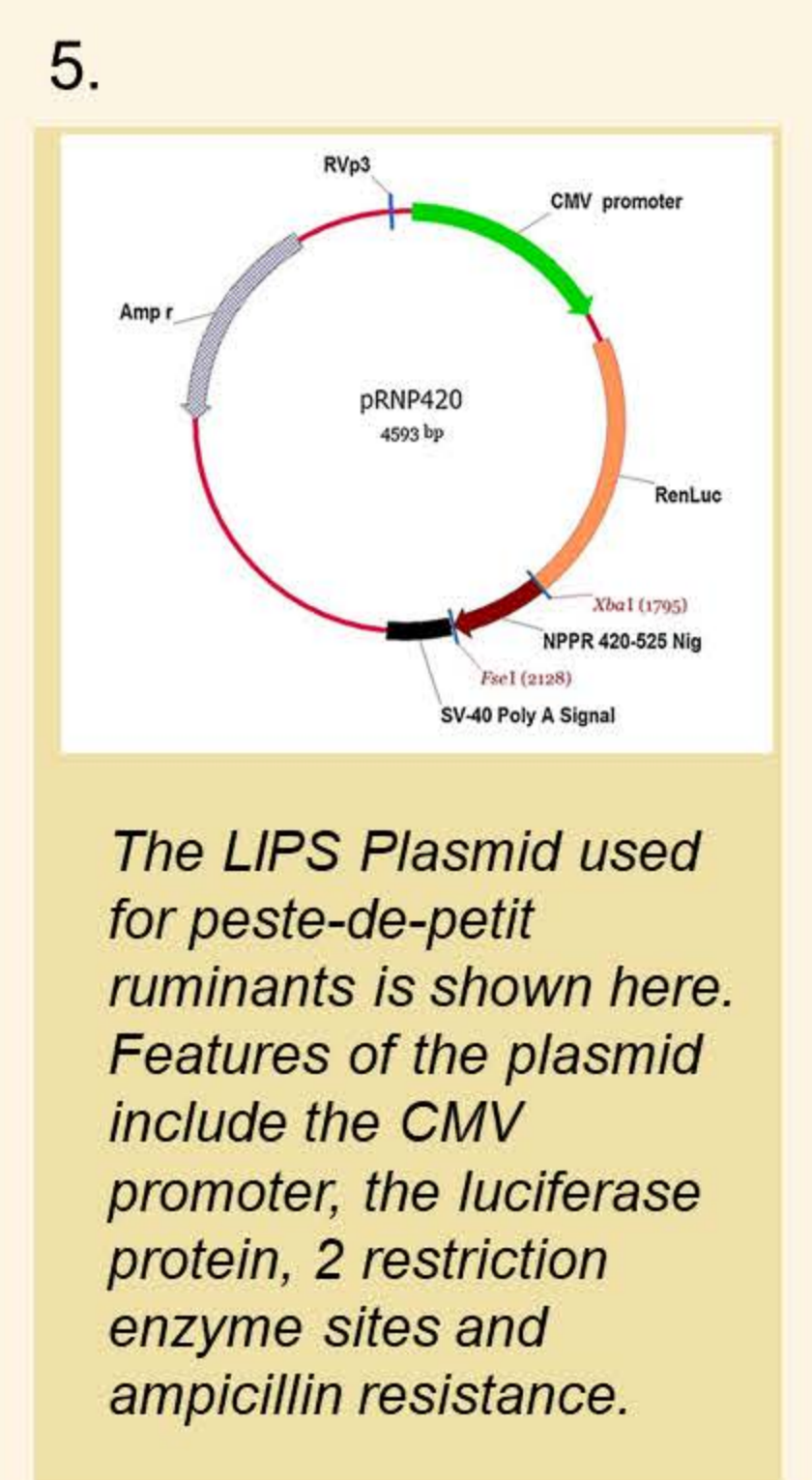
Plasmid construct

Six potential amino acid sequences were identified (Panel 7), chosen based on their sequence identity and scores using the specified algorithms. These plasmids will be constructed and purified for proof-of-concept testing within the LIPS. Panel 5 illustrates the structure of the proposed plasmid.



The origin of the LASV sequences by year of collection and country of origin is shown for the 676 sequences used in the analysis.

24 of these were obtained from rodents (3.6%) with the remaining 652 samples obtained from humans.



7.

Epitope Number	Start Residue	End Residue	Method identifying suitable antigenicity	AA Identity
1	26	42	K-T & Bepipred-2	99%
2	119	125	K-T & Bepipred & Bepipred-2	94%
3	246	257	K-T & Bepipred-2	99%
4	305	314	K-T & Bepipred-2	100%
5	447	487	K-T & Bepipred-2	90%
6	500	535	K-T & Bepipred & Bepipred-2	96%

The 6 epitopes selected for further development within the LIPS LASV program

Conclusions

We have identified a suitable assay to describe the seroprevalence of *Lassa mammarenavirus* in rodents and peridomestic animals in West Africa. The antigenic target is derived from publicly available viral sequences across the LASV endemic zone and across time. We have used established bioinformatic techniques to guide epitope selection.