

Analysis summary

	WNV NS3	HIV-1 <i>env</i>	SARS-CoV-2 spike
Gene-wide episodic selection (BUSTED)	No	Yes	Yes
Branch-level selection (aBSREL)	No	Yes, three branches, including transmission	No
Site-level episodic selection (MEME)	Yes, 1 site	Yes, 8 sites	Yes, sites found depend on which branches are included

It is not unexpected that site-level positive results can occur when a gene-level test does not yield a positive result

- **Lack of power for the global test:** if the proportion of sites under selection is very small, a mixture-model test, like BUSTED, will miss it.
- **Model violations:** MEME supplies much more flexible distributions of dN/dS over sites; compared to alignment-wide 3-bit BUSTED distribution.
- **False positives at site-level:** our site-level tests have good statistical properties, but each positive site result could be a false positive; FWER correction would make site-level tests too conservative.
- **Summary:** gene-level selection tests need a minimal proportion of sites to be under selection to be powered; site-level tests should not be used to make inferences about gene-level selection.