Background Continued post-elimination monitoring is required to ensure sustainability of zero transmission of human African trypanosomosis (HAT) and to avoid re-emergence caused by potential remaining *Trypanosoma brucei gambiense* reservoirs (animal and/or human). Until now, no tool is able to attest or validate elimination. Increasingly, the serological immune trypanolysis test is being implemented in the decision algorithms to characterise parasitological unconfirmed CATT or RDT seropositive subjects. Therefore, we wanted to assess further the high specificity of immune trypanolysis.

Methods We first tested samples from domestic animals from a tsetse-infested area in Ethiopia, a country where no *T. b. gambiense* exists, but where bovine trypanosomosis is prevalent. Then, we tested cattle and human samples from the south-west of Burkina Faso, a historical focus of *gambiense* HAT that still shelters tsetse flies populations and animal trypanosomosis. Lastly, we were interested in testing human samples from active foci in Côte d’Ivoire and Guinea.

Results Our results showed zero trypanolysis-positive animals from Ethiopia while in the historical HAT foci in Burkina Faso, 4.89% (14/286) of cattle were trypanolysis-positive. In humans, zero samples over 729 were trypanolysis-positive in Burkina Faso, while the percentage of positives was 3.77% (44/1166) in Guinea, including 7 new cases diagnosed during the sampling and 1.3% in Côte d’Ivoire (8/598).

Conclusions Considering results from this study, we think that trypanolysis test, confirmed to be a very specific test in human, can be a tool able to certify HAT elimination in a given area. It also suggests that the risk of the reintroduction of *T. b. gambiense* in Burkina Faso is real, especially in the southwest which shelters a high density of tsetse populations, in addition to the possible presence of *T. b. gambiense* in domestic animals. However, further studies on the specificity of the trypanolysis test regarding *T. b. gambiense* in animals should be conducted.