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Development of a SHERLOCK molecular diagnostic toolbox for the detection of trypanosomatid parasites

New diagnostic tools with highly specific and sensitive detection are needed as we move towards control or elimination of a number of neglected tropical diseases. We have adapted SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) for the detection of different species of trypanosomatid parasites, including human and animal African trypanosomiasis (HAT and AAT), as well as American trypanosomiasis (Chagas disease). SHERLOCK uses CRISPR-Cas13a to detect the presence or absence of RNA in a biological sample, allowing for the detection of an active infection. We have now developed (1) an 18S Pan-trypanosomatid SHERLOCK assay, capable of detecting the presence of any trypanosomatid parasites, (2) an 18S Pan-*Trypanozoon* SHERLOCK, (3) an 18S *T. congolense* SHERLOCK, (4) an IFX *T. vivax* SHERLOCK, and (5) an 18S *T. cruzi* SHERLOCK assay, which can discriminate between species of the same family with high specificity in a single sample. This methodology allows us to detect the presence of parasites in skin biopsies as well as in blood samples, at concentrations ranging from 0.5 to less than 0.05 parasites/µL according to the target. Furthermore, in order to adapt this technique to points of care, we are optimising an alternative nucleic acid extraction, and modifying the reaction conditions to reduce the cost, equipment needed, pipetting steps, and time to read-out.