SHERLOCK4HAT: a new CRISPR-based tool for Human African Trypanosomiasis diagnosis

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Human African Trypanosomiasis (HAT) is a debilitating, and often fatal, neglected tropical disease, caused by two subspecies of *Trypanosoma brucei* (T. b.) - *T. b. gambiense*, responsible for 98% of cases, and *T. b. rhodesiense*, accounting for the remaining 2%. Due to a concerted effort over the past 20 years, HAT is now approaching elimination, with the number of cases reported in 2019 dropping below 1,000. In this context, gambiense HAT was targeted for elimination as a public health problem by 2020 and sustainable elimination of transmission (zero cases) by 2030. These efforts are at risk to be undermined as the current field-based HAT diagnostic tools may lack the sensitivity and specificity required to meet the harsh constraints imposed by the WHO in the HAT elimination phase. We have adapted the recently developed Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) for the detection of *Trypanosoma* parasites and optimized the methodology for mass screening, surveillance of active infections and point of care testing. SHERLOCK is a CRISPR-based approach that relies on the collateral RNase activity of Cas13 upon target activation. With our assay, we are able to distinguish between three *T. brucei* subspecies without cross-reactivity and with sensitivity of 0,1 parasite/uL using *in vitro* as well as *in vivo* experimental and field isolated samples. We are currently able to detect parasitaemia lower than 100 parasites/mL in simulated human infections. We are now optimizing one-tube reactions coupled to a lateral flow assay making this a simple portable diagnostic to be used at point of care.