**Objectives**:

*Paracoccidioides* is a genus of thermally dimorphic fungi that can cause paracoccidioidomycosis (PCM), a systemic infection endemic to Latin America. Conventional methods for diagnosing PCM include microscopy, serological testing, and molecular identification. To improve the diagnosis of paracoccidioidomycosis (PCM) we aim to develop a rapid, low-cost, accessible and easy-to-perfom loop-mediated isothermal amplification (LAMP) assay.

**Materials & Methods:**

Nineteen *Paracoccidioides* strains, including *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*, and clinical material from 6 patients with confirmed PCM were used to develop the LAMP assay. Mitochondrial data of genome sequenced *Paracoccidioides* strains were collected from the NCBI Genome database to design various LAMP-assays using the NEB LAMP design tool (New England Biolabs, Ipswich, MA, USA). To test the specificity, 36 clinically relevant fungal pathogens were included. A gBlock (IDT, San Diego, CA, USA) was designed to determine the technical limit of detection. To enhance ease-of-use and improve specificity in result interpretation a lateral flow assay (LFA) was developed to complement the LAMP reaction using the ‘LFA starter pack’ (Milenia Biotec, Gießen, Germany).

**Results**:

Among the mitochondrial genes tested, the *ATP6* gene was found to be the most promising target to further test the foreseen LAMP-assay, both in terms of specificity and sensitivity. To further enhance ease-of-use and interpretation of the result, the LAMP-assay was transformed into an LFA assay using biotin labelled primers. This combined approach (LAMP-LFA) demonstrated a high diagnostic specificity with no false positives in the specificity panel, or false negatives in the set of *Paracoccidioides* strains. Moreover, the clinical samples from 6 PCM patients became all positive in the LAMP-assay and the LAMP-LFA-assay. The technical limit of detection of the LAMP-LFA-assay was found to be ≥1.000 copies of the target gene per reaction.

**Conclusions**:

LAMP-LFA is a technique with high specificity and sensitivity for the detection of *Paracoccidioides*. Due to its simplicity, speed and low-cost the technique represents a promising alternative to current techniques particularly in low-resource settings.