Point of Care Diagnostics for Cutaneous Fungal Infections

Katherine Bradley, Julia Hoffman, Fiona McEnany, Christine Hodge, Brian Simmons MD, Matthew Hayden MD

Abstract

Given the rising costs of care for dermatophytosis, paired with the limitation of low sensitivity and time-intensive clinical tests, there is a demand for rapid, cost-efficient, and accurate diagnostic alternatives. In this study, we developed a clustered regularly interspaced short palindromic repeats (CRISPR) Cas12a-based test using the highly conserved dermatophyte gene, Topoisomerase II (TOP II), which has been used to identify dermatophytes at the species level[1]. The results of Cas12a cutting of species-specific TOPII target DNA were visualized by the naked eye with lateral flow strips (LFA). We also introduced an additional rapid dermatophyte identification method using colorimetric loop-mediated isothermal (LAMP) detection. In the presence of a specific dermatophyte species, this assay amplified a target region of the TOP II gene, yielding a pH-induced color change indicative of a positive test. The question of scaling these identification methods to a pan-dermatophyte test was also explored, culminating in the creation of a pan-dermatophyte TOP II consensus sequence from which CRISPR crRNA guides and LAMP primers can be derived for streamlining future point of care testing and research applications.

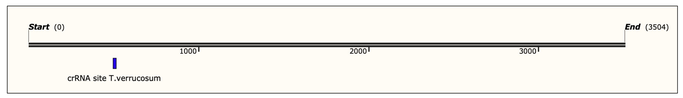
Introduction

Cutaneous dermatophyte infections are a growing medical condition in dermatological settings; It is estimated that up to 25% of the world's population suffers from dermatophytosis[2]. Despite its prevalence, clinical diagnostic testing for dermatophytosis relies on direct microscopic exam (DME) or fungal cultures. DME is limited by its ability to determine fungal species, and fungal cultures require 3 weeks for a result. Moreover, fungal cultures have been shown to have a sensitivity of 50% when DME and PCR test were positive[3].

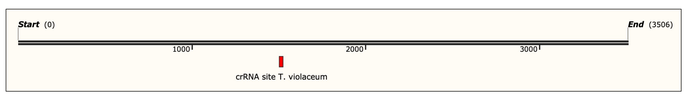
When considering systemic anti-fungal treatment, the American Academy of Dermatology recommends dermatophyte diagnostic testing because there are rare but serious side effects associated with these treatments. The cost of testing and treatment for onychomycosis alone in Medicare patients was over 27 million dollars in 2016 and increases every year[4]. Given the lack of sensitivity and efficiency of current diagnostic modalities, there is a need for an accurate, inexpensive, and rapid dermatophyte diagnostic test, both in the clinic and research labs.

Methods

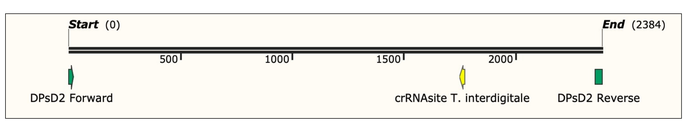
Partial sequence of TOP II gene with mapped CRISPR guide cutting sites for three dermatophyte species



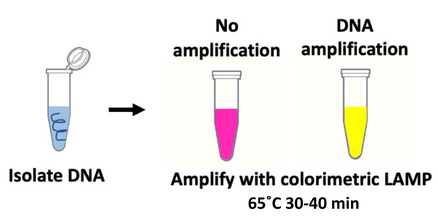
Trichophyton verrucosum **TOP II**



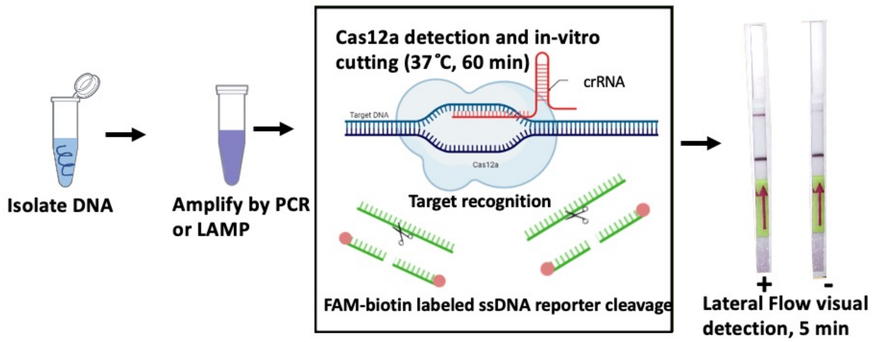
Trichophyton violaceum **TOP II**



Trichophyton interdigitale **TOP II**



Workflow of LAMP detection of dermatophytes. Isolated dermatophyte DNA can be amplified by colorimetric LAMP for a rapid one-step diagnostic test. The assay utilizes a visible pH indicator that changes the reaction from pink to yellow in the presence of proton production due to DNA polymerase activity, indicating DNA amplification.



Workflow of Cas12a identification of dermatophytes. Isolated DNA from dermatophytes can be amplified by PCR or other rapid PCR methods. The amplified target DNA is then introduced to Cas12a pre-incubated with the target-specific guide RNA (crRNA), resulting in double stranded DNA cleavage. Cas12a nuclease, now activated, proceeds to cut the FAM-biotin labeled single stranded DNA reporters. This reporter is detected by downstream lateral flow assay (LFA), indicating the presence of the target DNA.

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Results

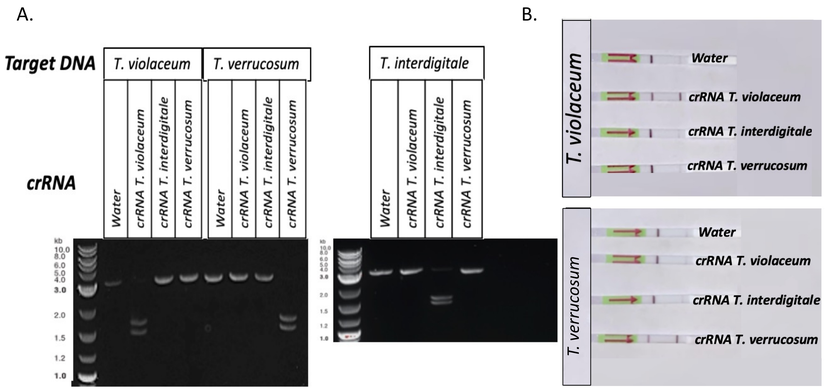


Fig. 1 Dermatophyte oligonucleotide identification using Cas12a-crRNA. Cas12a pre-incubated with species-specific crRNA guides were introduced to three manufactured dermatophyte oligonucleotide targets (IDT, Newark, NJ). Target cleavage was determined by lateral flow assay and confirmed with gel electrophoresis **A)** *T. violaceum*, *T. verrucosum*, and *T. interdigitale* oligonucleotides were cleaved by their respective Cas12a-crRNA complexes, showing Cas12a-crRNA specificity by gel electrophoresis. **B)** Lateral flow assays detected oligonucleotide target cleavage by CRISPR Cas12a-crRNA for all three dermatophyte species in a specific manner.

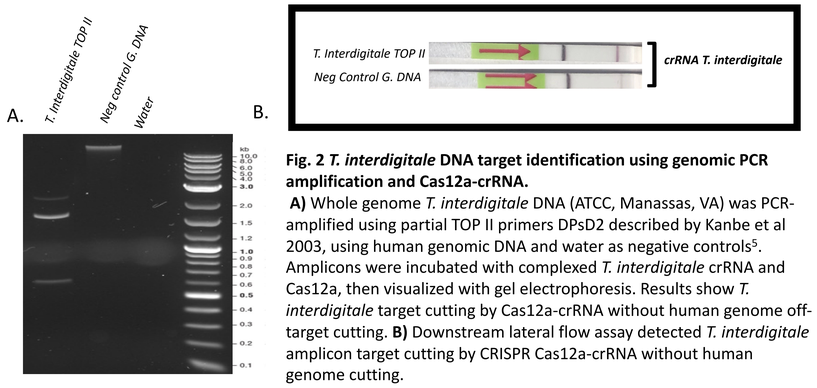
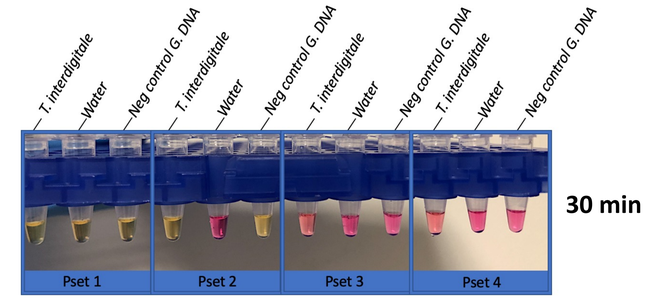


Fig. 2 *T. interdigitale* DNA target identification using genomic PCR amplification and Cas12a-crRNA.
A) Whole genome *T. interdigitale* DNA (ATCC, Manassas, VA) was PCR-amplified using partial TOP II primers DPsD2 described by Kanbe et al 2003, using human genomic DNA and water as negative controls[5]. Amplicons were incubated with complexed *T. interdigitale* crRNA and Cas12a, then visualized with gel electrophoresis. Results show *T. interdigitale* target cutting by Cas12a-crRNA without human genome off-target cutting. **B)** Downstream lateral flow assay detected *T. interdigitale* amplicon target cutting by CRISPR Cas12a-crRNA without human genome cutting.



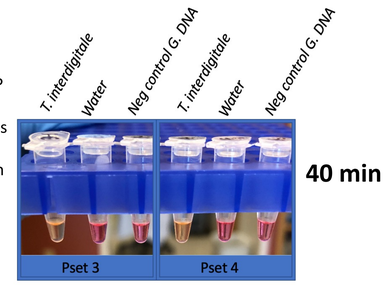


Fig. 3 LAMP amplification and detection of genomic *T. interdigitale* DNA. Four different LAMP primer sets (Pset 1-4) were screened for amplification of *T. interdigitale* TOP II target DNA as compared to human genomic DNA and water as negative controls. Primer sets 1 and 2 showed high non-specific amplification at 30 minutes. Primer sets 3 and 4 amplified *T. interdigitale* target DNA with a longer incubation period of 40 minutes.

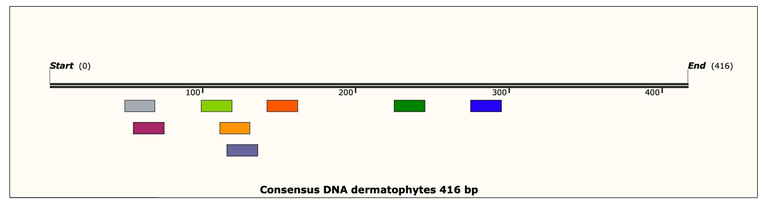


Fig. 4 TOP II consensus sequence for 122 dermatophyte strains with mapped CRISPR crRNA sites. The variations in the TOP II gene were analyzed for 122 dermatophyte species using GENBANK (NCBI, Bethesda, MD) for genomic sequences and Clustal Omega (RRID:SCR_001591) for percent identity analysis. The consensus sequence was determined by a 50% conservation cutoff, and potential CRISPR crRNA guide sites were determined by Benchling (RRID:SCR_013955).

Summary/Conclusions

In this study, we presented a CRISPR-Cas12a-based assay and LAMP assay that can identify dermatophytes at the species level and potentially the family level.

- Lateral flow assays and gel electrophoresis showed Cas12a-crRNA cutting of three species of dermatophyte target oligonucleotides with high specificity.
- Amplification of TOP II from isolated whole genome *T. interdigitale* DNA was performed by PCR with successful downstream Cas12a-crRNA cutting and detection by LFA and gel electrophoresis. Next steps include studying more rapid methods of PCR-amplification of crRNA target regions.
- One-step detection of target DNA of *T. interdigitale* was explored using colorimetric LAMP assays, which demonstrated that primer sets 3 and 4 identified *T. interdigitale* without off-target amplification. Next steps will include confirmatory gel electrophoresis of Cas12a cleavage of *T. interdigitale* and further specificity testing.
- A pan-dermatophyte consensus sequence was derived from 122 dermatophyte strains and potential crRNA sites were developed. Next steps will include screening the CRISPR guides against the major dermatophyte genera for sensitivity and specificity.

These results show two novel diagnostic tests for dermatophytes that can be used in a clinical or research setting for rapid detection of acute dermatophyte infections at a species level, and with further research, at a family level. Future studies could expand species-level identification of clinically relevant dermatophytes with a greater breadth of CRISPR-based or colorimetric LAMP-based assays. It would also be valuable to use the dermatophyte consensus sequence to develop a pan-dermatophyte CRISPR-based or a LAMP-based assay as an efficient point of care diagnostic test.

Acknowledgements

Geisel School of Medicine, Dartmouth-Hitchcock Medical center, Matthew Hayden MD, Brian Simmons MD, Christine Hodge

References

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