Development of in-house DNA extraction method for *Trypanosoma evansi* from dried blood samples on FTA cards

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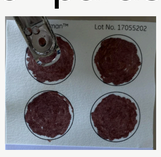
BACKGROUND

Surra affects animal health worldwide, including in Thailand. PCR is a key diagnostics but field application remains limited due to transportation fresh blood samples and the high cost of commercial extraction kits. Dried blood spots (DBS) offer alternative, but efficient and cost-effective extraction method is essential to large-scale surveillance.

METHODS

Samples: Serial dilutions of *T. evansi* isolate (NIAH,thailand) in confirmed negative bovine blood (10^6-10^1 parasites/mL) > spotting on FTA cards



DBS ; 3 x 3-mm punches

DNA extraction methods: commercial kit vs (1) SDS–Proteinase K; (2) Proteinase K-Chelex; (3) SDS-TE (95°C); (4) SDS-TE (room temperature); (5) TE-Chelex; and (6) TED10(TE+10% DMSO)-Chelex

PCR: TBR1/TBR2 primers

LOD determination: 3 replicates

RESULT & DISCUSSIONS

Extraction Method	LOD (parasites/mL)
Commercial Kit	10^3
TE-Chelex (In-House)	10^3
TED10-Chelex	10^3
SDS-Proteinase K	10^6
Proteinase K-Chelex	10^6
SDS-TE (95°C)	Not detectable
SDS-TE (Room Temp)	Not detectable

The **TE–Chelex** method achieved a limit of detection of **10^3** parasites/mL, which was comparable to that of the commercial extraction kit. The **TED10-Chelex** method showed **similar sensitivity** but required more complex processing steps. The **SDS–Proteinase K** method and the **Proteinase K–Chelex** method showed lower sensitivity, with LOD values of **10^6** parasites/mL. **The SDS–TE** methods at both temperatures did not yield detectable amplification under the conditions tested.

CONCLUSION

in-house TE- Chelex extraction method

- offers comparable sensitivity to commercial kits.
- is reliable alternative for DNA extraction from DBS samples.
- provides simple workflow while using less reagents and materials.

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