UNIVERSITÄTSMEDIZIN GÖTTINGEN : UMG

VALIDATION OF A NON-INVASIVE DIAGNOSTIC TEST FOR PRION DISEASES USING TEAR FLUID

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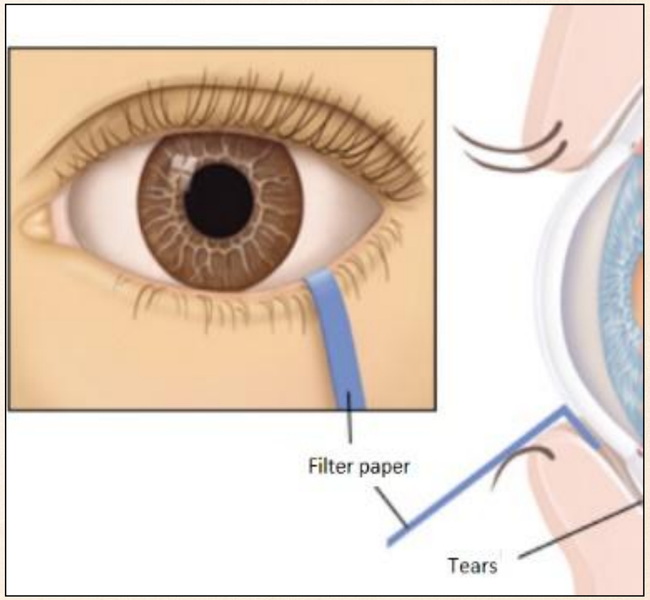
NEW APPROACH: DETECTION OF PRIONS IN TEARS VIA RT-QUIC AGGREGATION ASSAY

Lumbar punctures are **invasive showing side-effects**:

- Frequent: Headache, nausea, and local pain.
- Very rare: Meningitis, potential spinal mass hemorrhage, brain stem entrapment.

- **Detection of PrP^{Sc} in less invasive body fluids such as** Tear fluid (TF):
- Advantages: easy to perform:

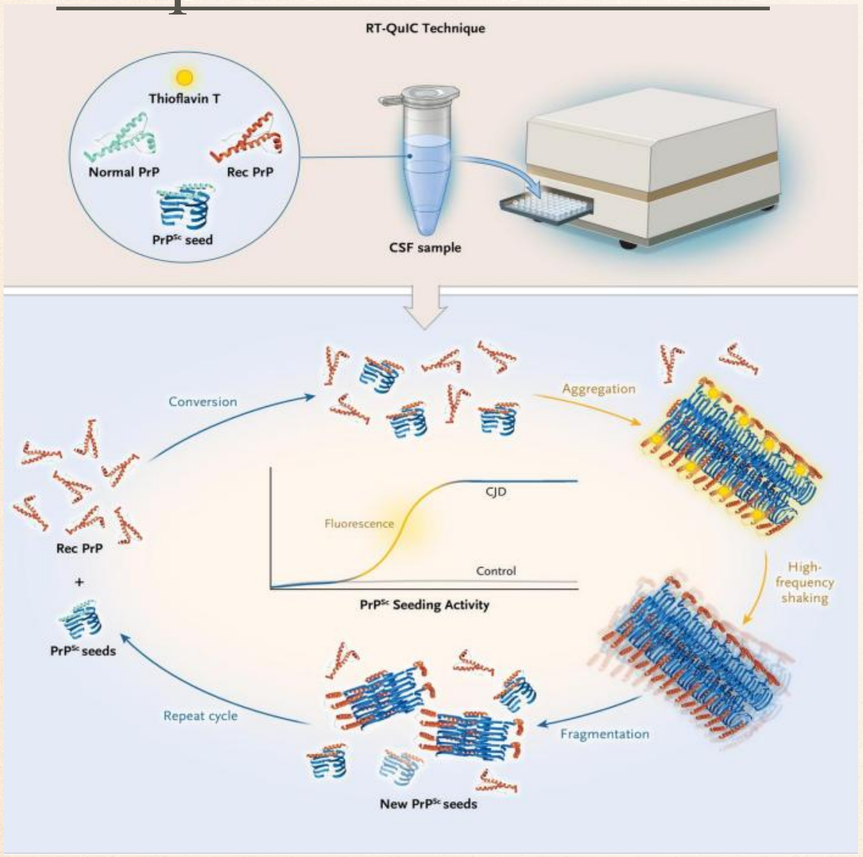
1) Place a 5 mm wide strip of filter paper into the outer canthus of the eyelid.
2) Remove the filter paper after 5-10 minutes
3) Extraction of proteins and detection of prions by RT-QuIC (protein aggregation assay



Schirmer-Test

REAL-TIME QUAKING-INDUCE CONVERSION (RT-QUIC)

Amplification of Prions



Zerr I, 2022 NEJM

Established for CSF-Diagnostic

RT-QuIC	Sensitivity	Specificity
sCJD	85-91%	99-100%
FFI	28%	100%

Schmitz M. *et al*., Brain 2022

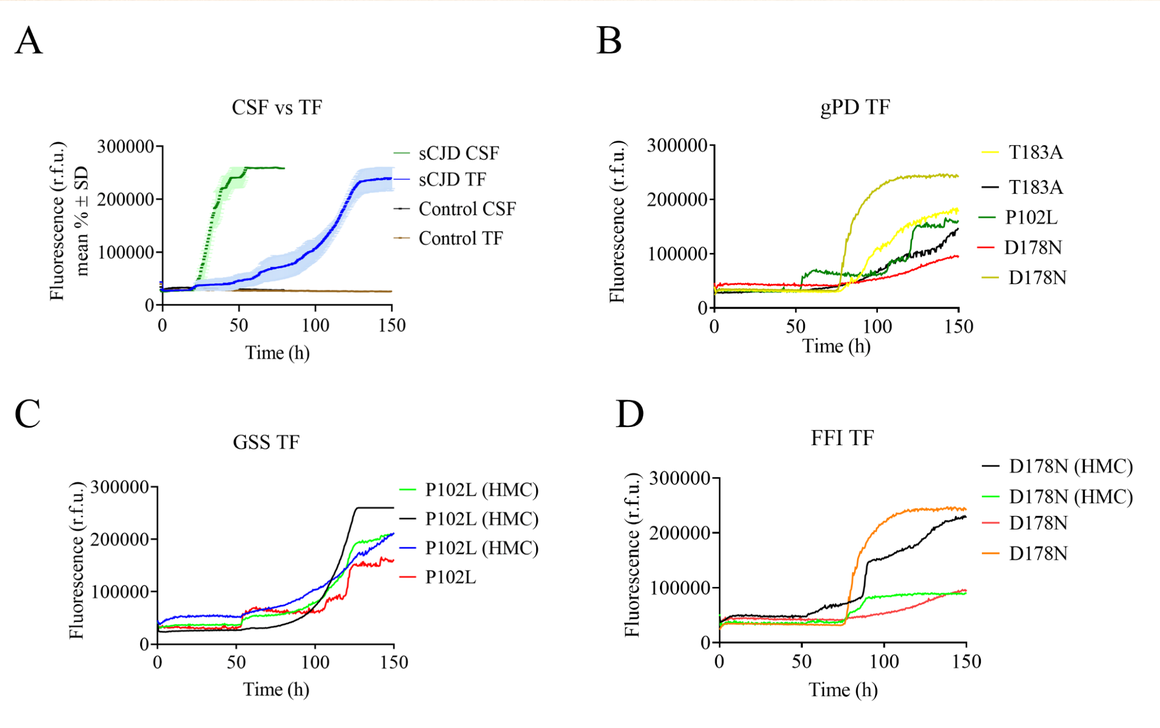
Schmitz et al., 2023 NEJM

FINDINGS IN THE PROJECT

1) Pre-analytic studies confirmed the **stability& reproducibility** of TF-RT-QuIC
2) Definition of the **minimal amounts of TF** (>10µL) for a reliable detection
3) Validation of the **diagnostic accuracy** of TF RT-QuIC in a second cohort
4) Longitudinal evaluation of TF RT-QuIC as a dynamic marker during the course of disease in prion disease patients
5) Longitudinal evaluation of TF RT-QuIC in healthy PRNP mutation carriers, regarding its potential ability to **predict disease onset**

Parameter	Recommendation
Amount of TF on the Strip per reaction	15-20 mm (>10 µL)
Duration of the TF RT-QuIC	150h
Software settings of the TF-QuIC	1 min double orbital shaking at 700 rpm and 1 min incubation
Definition of a positive reaction	> 50% positive reactions
Storage of the samples	Stable for at least one week at RT and against 12 cycles of freezing and thawing
Kind of recombinant substrate	Highly sensitive FL Hu E200K
Collection of TF	Schirma method

DIAGNOSTIC ACCURACY IN TF RT-QUIC



	1st cohort	2nd cohort	Total Sensitivity
sCJD	**13/15 (87%)**	**14/17 (82%)**	**27/32 (84%)**
Genetic prion diseases	4/5 (80%)	3/5 (60%)	7/10 (70%)
FFI	2/2 (100%)	1/2 (50%)	3/4 (75%)
GSS	1/1 (100%)	2/3 (67%)	3/4 (75%)
T183A	1/2 (50%)	-	1/2 (50%)
Non-prion diseases[3*]	0/68 (0%)	1/116 (99.1%)	1/184 (99.5%)

Correia*, Schmitz* et al., 2025

- Lower signal intensity in TF compared to CSF
- Signal detection in healthy mutation carriers (HMC) possible
- Diagnostic sensitivity lower in TF

IMPLICATIONS AND FUTURE WORK

→ **TF-QuIC is particularly useful when a lumbar puncture is not possible or during longitudinal follow-up studies conducted at multiple time points**

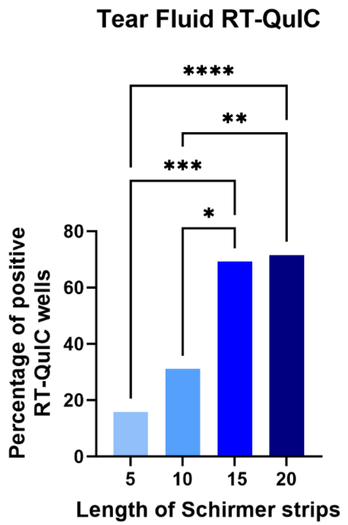
Outlook:

1) Increasing the numbers and further optimization of the TF QUIC
2) Analysis of pre-symptomatic mutation carriers: Can the TF RT-QuIC predict the disease onset?
3) Proteomic Studies: Detection of further disease relevant proteins in TF

ACKNOWLEDGEMENT

- **Michale H. Cole Memorial Research Grant** (contributed by Jeanne Cole)
- **Lynda Morris Memorial Grant** (contributed by David Morris and Family)
- **Tom Stivison Memorial Research Grant** (contributed by Sandra Stivison)
- **Families** of the CJD Foundation and **Strides** for CJD

A



B

µl	TF strips (mm)
1	< 1mm
1,25	1mm
2,5	4mm
5	9mm
10	19mm
20	32mm

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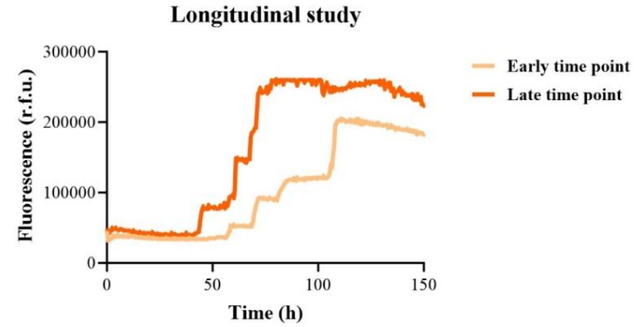

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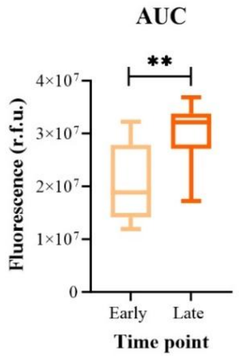

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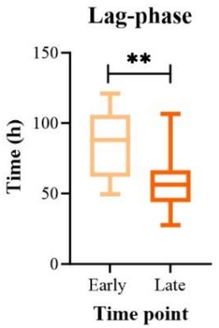
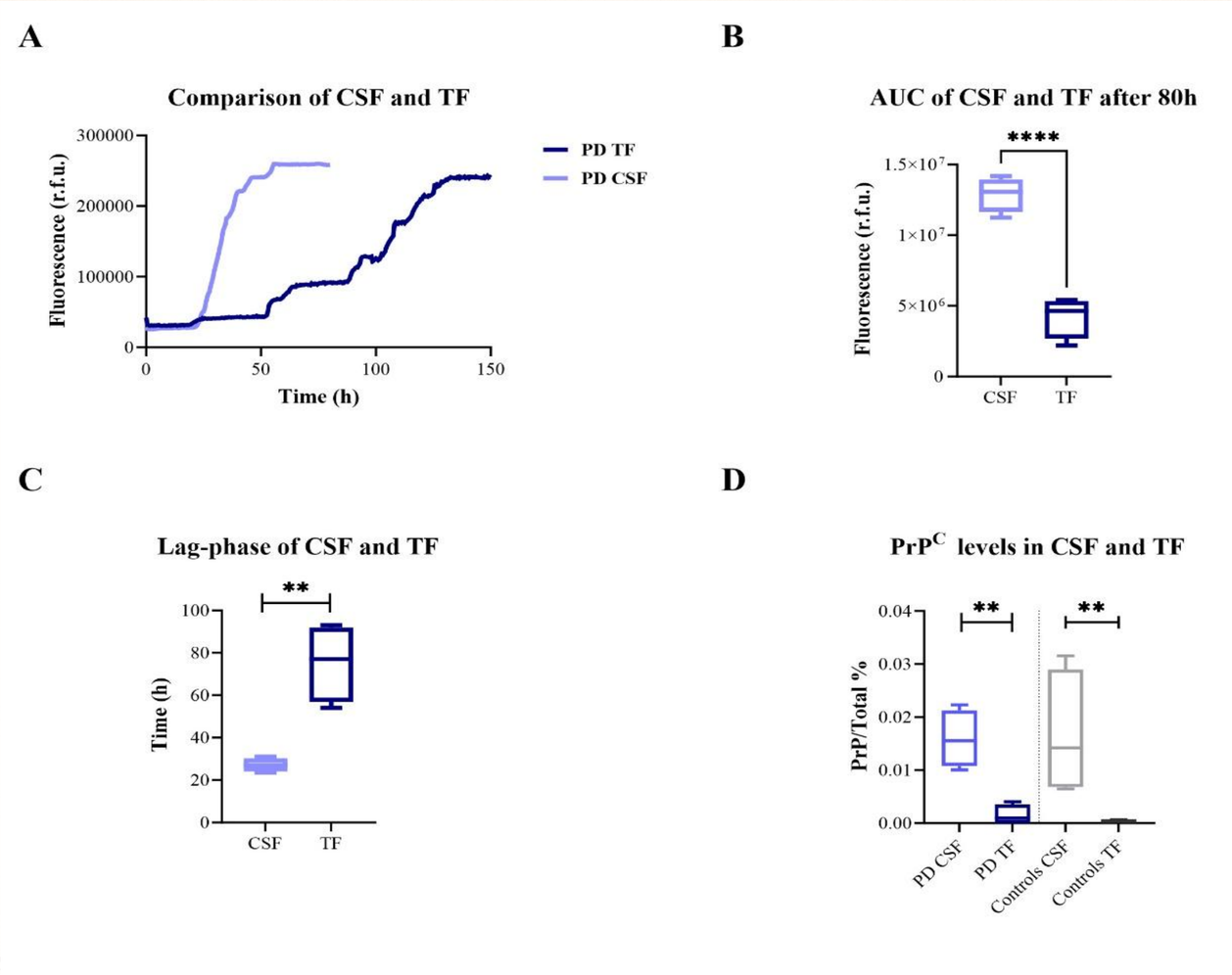


Comparison between CSF and TF



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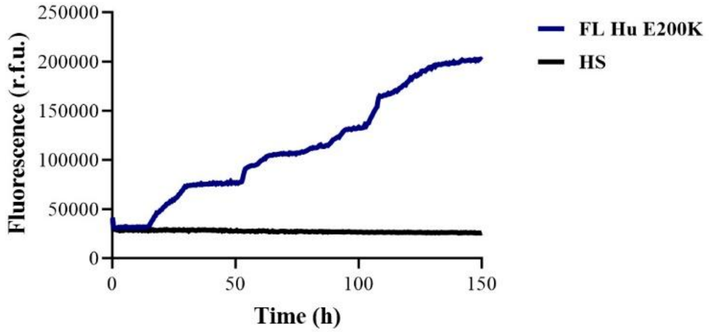

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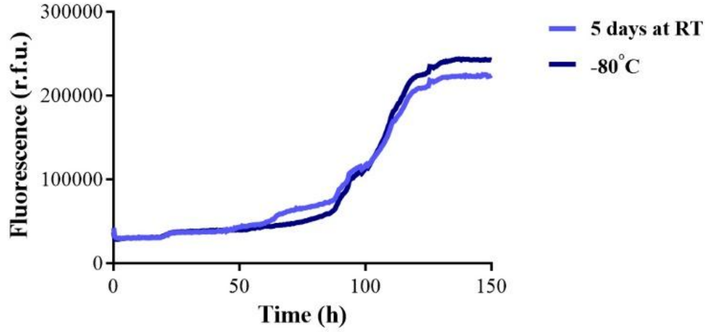
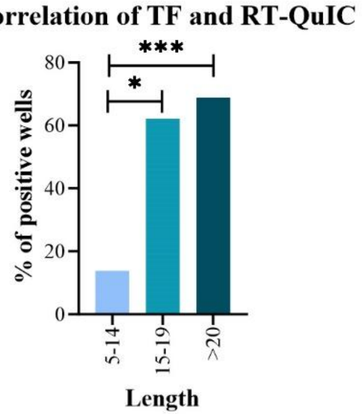


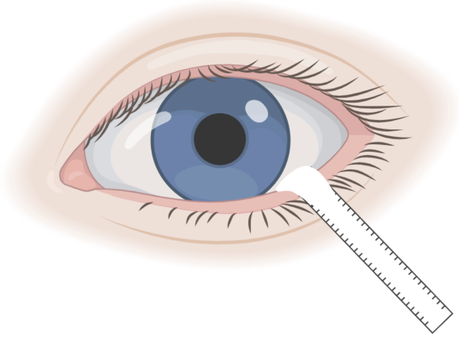
C

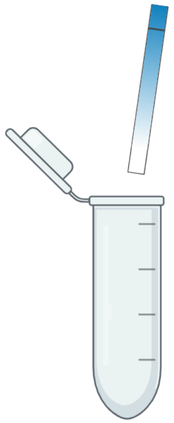
Volume TF in (µL)	Wetting length in (mm)
1.25	1
2.5	4
5	9
10	19
20	32

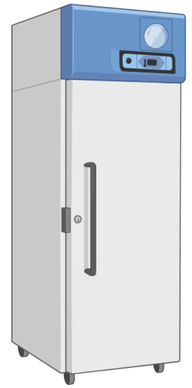
D



Tear fluid collection workflow



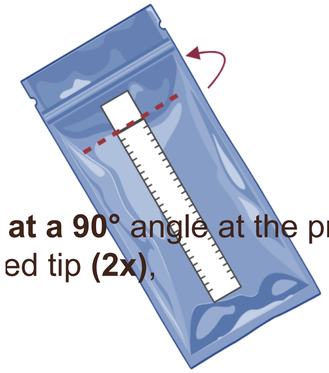




± 10 min
2x

4x

-80°C



at a 90° angle at the pre-ed tip **(2x)**,

2. Insert the folded end of the strip into the **lateral and middle third of the lower eyelid margin (2x),**

3. Instruct the patient to Keep the **eyes closed for at least 10 min,**

4. **Remove the strip** carefully without touching wetted portion,

5. Repeat the steps 1 to 4 one more time.

6. Store the tear fluid strips at -8°C.

Summary and Conclusion

Enhanced Prion Diagnostics

Low RT-QuIC Diagnostic Sensitivity in gPD

genetic prion diseases
(e.g. FFI (19% sensitivity))

Purification of different recPrP

FL Hu E200K
novel substrate

Enhanced CSF Sensitivity

Improving detection accuracy

Tear Fluid Detection

Enabling non-invasive sampling

High Diagnostic Sensitivity

CSF:
gPD 75% to 100% sensitivity
TF:
gPD 70% sensitivity