Toxichem Krimtech 2011;78(Special Issue):332

Quantification of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (CTHC) Equivalents in Urine using Instrumentally Read Gold-labeled Lateral Flow Immunoassays (IRGLFIA) in Order to Predict THC in Serum ≥ 1 ng/mL

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Key words: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, quantification, lateral-flow-immunoassay, THC, serum, § 24a.II StVG

Abstract

Aim: According to specificity and sensitivity data for THC detection in serum based on 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (CTHC) concentration in urine, quantitative measurement of CTHC equivalents in urine and knowledge of the CTHC concentration actually excreted in urine should help police officers to detect drugged drivers.

Methods: We have developed well-tailored lateral flow tests for CTHC in urine using a special nanoliter dot printer (P.I.A.-print) for highly precise test lines that is appropriate for quantitative reading of CTHC-concentrations in urine with an optoelectronic reader (P.I.A).

Results: The test results were in good correlation to GC-MS measurements of CTHC in urine and with CEDIA-DAU immunoassay (ThermoFisher) allowing a road side pilot study in order to show, that the above goal can be achieved. According to the 28 data pairs obtained until now, serum THC values of < 1 ng/mL occurred when urinary CTHC equivalents were around 150 ng/mL and not at higher values.

Discussion: Our measurement system is the first rapid testing system based on machine-read test strips that are precise enough for quantitative immunological measurements of urinary CTHC-Equivalents, which can be referred to a THC serum concentration ≥ 1 ng/mL.

Conclusion: Quantitative measurement of CTHC-equivalents in urine using well-tailored IRGLFIA and P.I.A. demonstrate enhanced possibilities in regard to roadside blood sampling decisions and is a more appropriate approach to roadside testing demands of the German police.

1. Introduction

For testing DUID according to the German laws § 24a.II StVG, police officers often use roadside rapid tests. In an earlier study, we first showed that the correct use of a twin test cassette (Check 24) with two visual "cutoffs", 50 and 150 ng/mL, can enhance the detection frequency of users with THC in serum ≥ 1 ng/mL by around 10 % up to 86% [1]. Based on this study, we have now calculated both, the specificity and the sensitivity of urinary GC/MS-CTHC concentrations for the detection of Δ9-tetrahydrocannabinol (THC) in serum ≥ 1 ng/mL using 211 road side sample pairs, as shown in Fig. 1 und 2. The plots show that the specificity (Fig. 1) increases with urinary CTHC concentrations reaching > 99 % at values > 500 ng/mL.

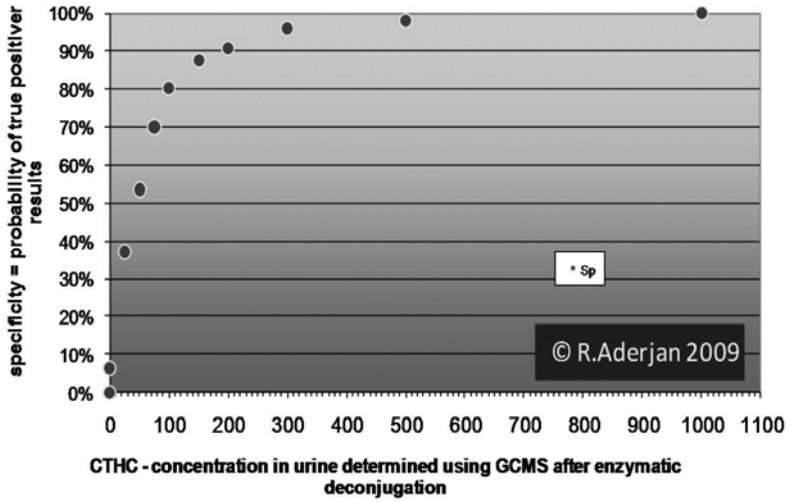


Fig. 1. CTHC-specificity plot. It shows how the frequency of true positive results (THC in corresponding serum sample ≥ 1 ng/mL) is related to the CTHC concentration in urine.

In contrast, the sensitivity plot (Fig. 2) shows how the number of missed cases with THC ≥ 1 ng/mL serum will increase when single qualitative test strips with cutoffs > 50 ng/mL or even > 150 ng/mL are used.

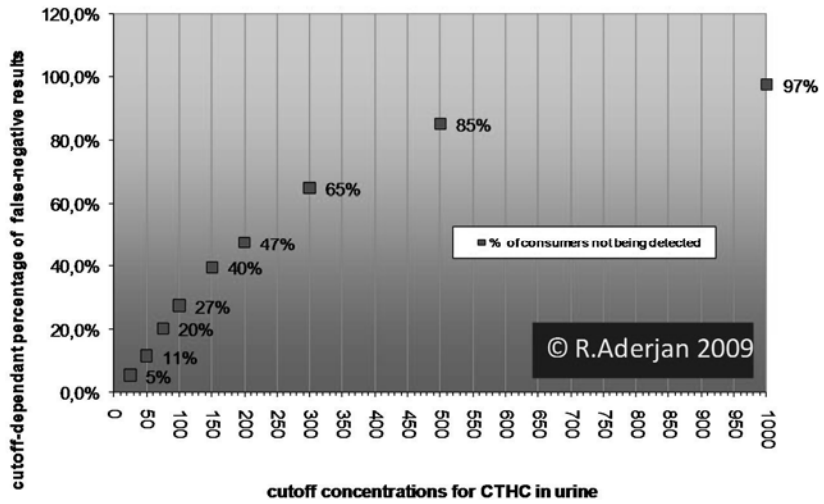


Fig. 2. CTHC - sensitivity plot for cutoff-dependant percentage of false-negative results (THC > 1 ng/mL in corresponding serum samples which may be missed, if the designated cutoff is used.

Accordingly, the use of machine-read lateral flow immunoassays for the measurement of urinary CTHC-equivalents ≤ 500 ng/mL of urine with a 50 ng/mL cutoff may help to predict a fined THC concentration in serum. We have developed an immunoassay for corresponding measurements. In comparison with other usually applied laboratory tests, the P.I.A measurement range is expanded upwards.

2. Material and Methods

Corresponding competitive IRGLFIA designed with highly accurate and appropriately homogenous lines of CTHC-BSA (Fitzgerald Ind. Int. North Acton, MA 01720 USA) were printed on a nitro-cellulose membrane using a PipeJet® printer (P.I.A.-Print) developed in co-operation with the Institute of Microsystem Techniques (IMTEK), University of Freiburg (Fig.3).

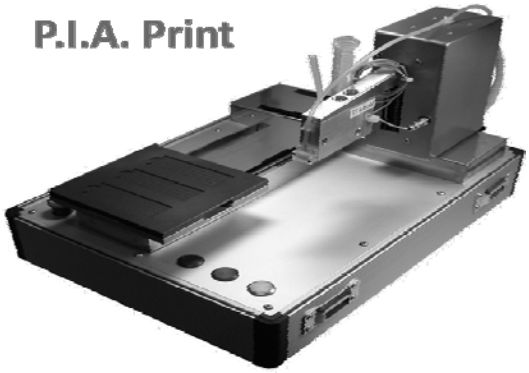
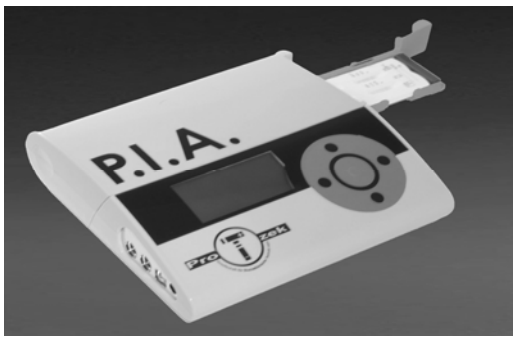


Fig. 3. PipeJet® printer (P.I.A.-Print).

Specific anti-CTHC-antibodies were also purchased from Fitzgerald. Quantitative readings of CTHC concentrations in urine were performed in appropriate dilution using a mobile opto-electronic reader equipped with lines scanning optical sensor and appropriate measurement software. (P.I.A.-Protzek GmbH, Fig. 4).



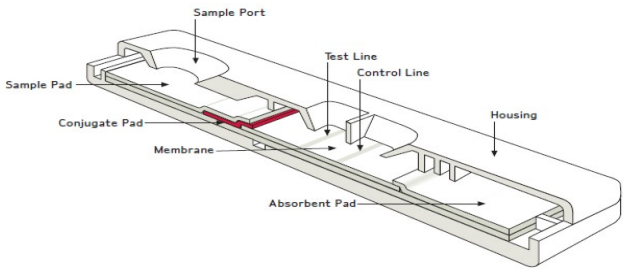


Fig. 4 and 5. Mobile opto-electronic reader equipped with lines scanning optical sensor and appropriate measurement software (P.I.A. - Protzek GmbH).

In Fig. 4 and 5, a typical standard test cassette, the P.I.A. reader and the test lines reading principle is shown: An optical sensor and lens system is moved along the test strip while scanning the reflected light and line intensity which depend on the analyt concentration. The concentration in a sample is read from a calibration curve related to the test strip batch. Anti-CTHC antibodies binding to CTHC-BSA test lines leads to a saturation curve which can be best fitted using the Rodbard function [2]. Batch-dependant calibration curves were fitted in the CTHC concentration range between 50 and 500 ng/mL. CTHC equivalents from 50 urine samples of drug users were estimated using IRGLFIA/P.I.A. and CEDIA-DAU immunoassay (ThermoFisher) and compared with GC/MS measurements after alkaline deconjugation. A pilot study is being conducted, using real samples and results obtained in practical forensic road side cases.

3. Results and Discussion

P.I.A. measurements show a good correlation to GC/MS measurements of deconjugated CTHC in urine. CEDIA-DAU and P.I.A. measurements are also in satisfactory agreement.

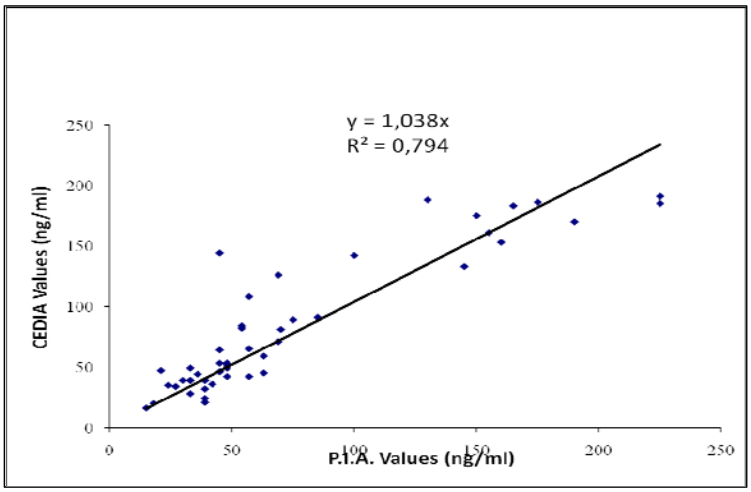


Fig. 6. Correlation data obtained from the comparison of urinary CTHC determined using CEDIA-DAU immunoassay and own well-tailored quantitative lateral flow immunoassays.

As shown in Fig. 6 and 7, the P.I.A. measurement range obtained with well-tailored precisely dot-printed lateral flow test allows to determine of CTHC-equivalents concentrations above 200 ng/mL and even above 1000 ng/mL, if needed. The whole measurement system is just as fast as the usual: immunoassay results that is obtained within 10-15 minutes entailing a maximum time window for proper readings of approximately 45 minutes.

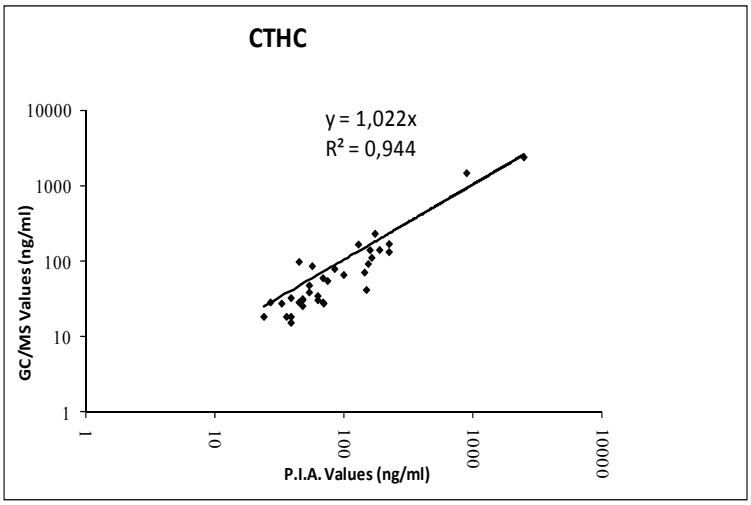


Fig. 7. Correlation data obtained from the comparison of urinary CTHC determined using GC-MS after alkaline deconjugation and our well-tailored lateral flow immunoassays.

As shown in Fig. 8, our pilot study presently comprises 28 police roadside urine samples and corresponding serum THC data. Corresponding data of CTHC in serum are also available though not shown in the graphs. According to the data pairs obtained until now, serum THC

values of < 1 ng/mL had occurred when urinary CTHC equivalents were around 150 ng/mL and not at higher values. As can be expected according to the above sensitivity and specificity measures, "outliers" should occur with decreasing probability if urinary CTHC is found above 250 ng/ mL. They may be regarded as false-positive in terms of a prediction of THC in Serum ≥ 1 ng/ mL. and consequent fines according to the § 24a.II StVG (of the German act).

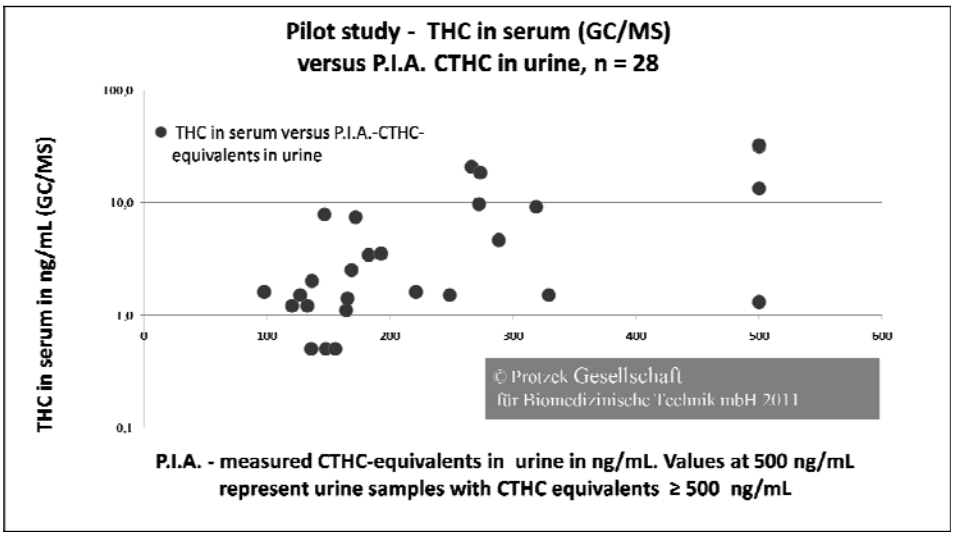


Fig. 8. Pilot study based on real roadside sample of cannabis users showing that serum THC values of < 1 ng/mL predominantly occurred when urinary CTHC equivalents were around or less than 150 ng/mL but not at higher values.

However, according to the above sensitivity data as shown in Fig 2, it must be taken into account, that the number of overlooked cases must increase, if any concentration limits are set (threshold values or cut-offs above 50 ng/mL) and drug influence monitoring is solely based on lateral flow tests. Hence, P.I.A. concentration reading can be regarded as a prognostic aid, powerfully complementing but not substituting professional road side experience in recognition of drugged drivers. Experienced testers keep in mind that cannabis-related effects strongly depend on individuals and using frequencies. The study will be continued.

4. Conclusion

Our measurement system is the first rapid testing system based on read test strips precise enough for quantitative immunological reading of urinary CTHC-equivalents which can be referred to THC in serum. Quantitative measurements of CTHC-equivalents in urine using well-tailored IRGLFIA and P.I.A. demonstrate superior possibilities in regard of roadside blood sampling decisions and should be a trendsetting helpful approach to roadside testing demands of the German police.

5. References

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