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**Manual**

# *IDK*® Psychotropic Medication 1 LC-MS/MS Kit

***For the in vitro determination of eleven tricyclic antidepressants in serum***

Valid from 2023-03-16

REF **KM6310**

Σ 300

+8 °C
+2 °C

IVD

CE

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## 1. INTENDED USE

The intended use of this device is to aid in the therapeutic drug monitoring (TDM) of several psychotropic medications (i.e. clozapine, norclozapine, nortriptyline, E-10-OH-nortriptyline, Z-10-OH-nortriptyline, amitriptyline, clomipramine, norclomipramine, imipramine, desipramine & zuclopenthixol) by determination of these medications in serum, performed by automated quantitative LC-MS/MS assay technology conducted by laboratory professionals.

## 2. INTRODUCTION

Therapeutic drug monitoring (TDM) is based on the assumption that there is a relationship between blood concentration and clinical effect (therapeutic improvement and adverse effects). It is also assumed that there is a concentration range of the drug that is characterised by maximum efficacy and maximum safety, the "therapeutic window" [1]. This Immundiagnostik LC-MS/MS assay includes amitriptyline, nortriptyline, clomipramine, imipramine and desipramine (including their metabolites) for tricyclic antidepressant (TCAs) as well as the antipsychotic clozapine (including the metabolite norclozapine).

The TCAs amitriptyline, nortriptyline, clomipramine, imipramine and desipramine are used to treat various types of depression, obsessive-compulsive disorder, neuropathic pain, nocturnal enuresis and for the prophylactic treatment of chronic tension headaches and migraine [2–5]. TCAs have marked interindividual pharmacokinetic variability and a narrow therapeutic window. Studies on the relationship between blood concentration and clinical improvement have confirmed this relationship for TCAs [1]. Systematic reviews and meta-analyses provided convincing evidence of a significant association between clinical outcomes and plasma concentrations for the TCAs, which are associated with a high probability of response [1,6–8].

TDM of amitriptyline, nortriptyline, clomipramine, imipramine and desipramine is strongly recommended in the consensus guidelines for TDM in psychiatry [1]. The guidelines state that reported drug concentrations are established and evaluated therapeutic reference ranges. Controlled clinical trials have shown beneficial effects of TDM, and reports on decreased tolerability or intoxications are present. TDM is therefore strongly recommended for dose titration at the start of the treatment, and for special indications, such as in patients with therapeutic failure, adverse events, drug-drug interactions, relevant comorbidities, altered CYP2D6 or CYP2C19 metabolic activity, and if nonadherence is suspected. For the TCAs, reference concentrations are based on literature and an overview of target concentrations can be found in several articles and the consensus guidelines for TDM in psychiatry [1,6-8]. Furthermore, the therapeutic range for amitriptyline and nortriptyline is stated in the summary of product characteristics (SPC) of these drugs [2,3].

Clozapine is indicated in treatment-resistant schizophrenic patients and in schizophrenia patients who have severe adverse reactions to other antipsychotic agents, including atypical antipsychotics. Clozapine is also indicated in psychotic disorders occurring in Parkinson's disease, in cases where standard treatment has failed. TDM of clozapine is advised in the SPC in certain clinical situations, such as when a patient ceases smoking or switches to e-cigarettes (altered metabolism of clozapine can lead to altered clozapine exposure), when concomitant medicines may interact and increase of decrease clozapine blood concentration, where poor clozapine metabolism is suspected, when a patient has pneumonia or other serious infection, and in the event of onset of symptoms suggestive of toxicity (adverse events) [9]. Furthermore, a high inter-patient pharmacokinetic variability of clozapine is seen [10]. This pharmacokinetic variability, in combination with a good correlation between clozapine blood concentrations and efficacy/toxicity makes TDM also useful at the start of clozapine treatment for dose titration, in case of an insufficient response to the treatment, in case of suspected non-adherence, and with the use of high clozapine doses [11–14]. TDM of clozapine is therefore strongly recommended in the consensus guidelines for TDM in psychiatry [1].

Besides for TDM, measuring blood concentrations of TCAs and antipsychotic drugs is helpful in the management of an intoxication with one of these drugs [15–17]. It is known that these drugs have a small therapeutic window and signs of toxicity are not always easily recognized purely on clinical grounds. Therefore, measuring blood concentrations will help to identify intoxications and guide in clinical patient management.

## 3. MATERIAL SUPPLIED

| Cat. No. | Identifier | Kit components | Quantity |
|---|---|---|---|
| KM6310 | AUTOWASH | Autosampler wash solution | 1 x 1 000 ml |
| | CAL1–6 | Calibrators 1–6; lyophilised (see product specification for concentration) | 2 vials (à 500 µl) per level |
| | CTRL1–3 | Controls 1–3; lyophilised (see product specification for concentration) | 3 vials (à 500 µl) per level |
| | PREC | Precipitation solution (contains internal standard) | 3 x 100 ml |
| | MOPHAA | Mobile phase A | 1 x 500 ml |
| | MOPHAB | Mobile phase B | 1 x 500 ml |

For reorders of single components, please use the catalogue number followed by the identifier without space as product number.

## 4. MATERIAL REQUIRED BUT NOT SUPPLIED

The following accessories are required for the *IDK*® Psychotropic Medication LC-MS/MS application (not included in the kit):

- Ultrapure water*
- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Centrifuge 10 000 *g* (at least)
- Vortex mixer or microtiter plate shaker
- Standard laboratory disposable plastic reagent tubes (e.g. 1.5 ml)
- LC-MS/MS system and LC-MS vials

*Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696/LC-MS grade), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

The following accessories for the *IDK*® Psychotropic Medication LC-MS/MS application can be ordered seperately at Immundiagnostik AG:

- UPLC column (KM6310SP)
- all single components

Please ask for our single component price list. Please contact us for customized inquiries.

## 5. PREPARATION, STORAGE AND STABILITY OF REAGENTS

**Note:** Please unpack the kit components from the transport packaging immediately upon receipt and follow the instructions for storage conditions printed on the product labels. In general, all components should be stored protected from light, dry and at the specified storage temperature.

All components are for LC-MS/MS use only, components may also contain other ingredients than those listed as active ingredients below which might influence the measurement. All declared stabilities are only valid in case of no bacterial contamination.

### *Calibrators and controls*

**Handling:**

Always remove the cap and rubber plug carefully (in order to avoid loss of content).

Reconstitute the calibrators and controls as follows:

- Reconstitute each calibrator and control with exactly 500 µl distilled or deionised water and incubate for 15 min at room temperature.
- Next, mix the component thoroughly to make sure that all dry material has dissolved; do not shake too vigorously to avoid foam formation.
- Handle the prepared component as a patient sample during the test procedure.

**Stability and storage:**

| | | |
|---|---|---|
| Before reconstitution: | 2–8 °C | Until expiry date printed on the product label. |
| After reconstitution: | 2–8 °C | 48 hours |
| After reconstitution: | -20 °C | 2 weeks |

### *Precipitation solution*

**Handling:**

The components are liquid and ready for use.

**Stability and storage:**

| | |
|---|---|
| Store at 2–8 °C | After first opening the component can be used for 4 weeks if closed and stored at 2–8 °C. |

### *Mobile phases A and B*

**Handling:**

The component is liquid and ready for use.

**Stability and storage:**

| | |
|---|---|
| Store at 2–8 °C | After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C or 2 weeks on the UHPLC. |
| Store at RT | Before first opening the component can be stored for 12 weeks at room temperature. |

### *Autosampler washing solution*

**Handling:**

The components are liquid and ready for use.

**Stability and storage:**

| | |
|---|---|
| Store at 2–8 °C | After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C or 2 weeks on the UHPLC. |

## 6. STORAGE, STABILITY AND PREPARATION OF SAMPLE

**Storage and Stability**

Use human serum. Avoid freeze-thaw cycles.

Serum samples can be stored: 3 months at -20 °C.

**Sample preparation**

| | 1.5 ml reaction tube | 96-well plate |
|---|---|---|
| 1. | Pipet 25 µl sample, CAL or CTRL into one reaction tube respectively well. | |
| 2. | Add 1 000 µl PREC. | |
| 3. | Vortex the tube for at least 30 s. | Shake the plate for 15 min. |
| 4. | Centrifuge for 5 min at 10 000 *g* (or more). | |
| 5. | Transfer the supernatant into a suitable LC-MS vial or 96-well plate for the LC-MS autosampler. | |
| 6. | Injection in the LC-MS/MS (see application note). | |

## 7. LC-MS/MS METHOD

Please refer to the application note or contact lcms@immundiagnostik.com for the LC-MS/MS method parameters.

## 8. EXAMPLES OF CHROMATOGRAMS

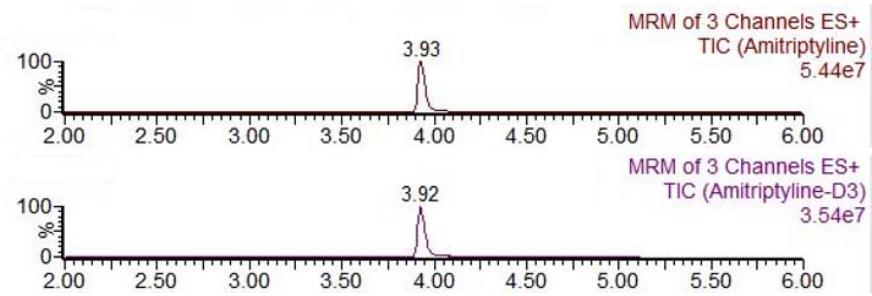


Fig. 1: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top) and internal standard (bottom) of amitriptyline.

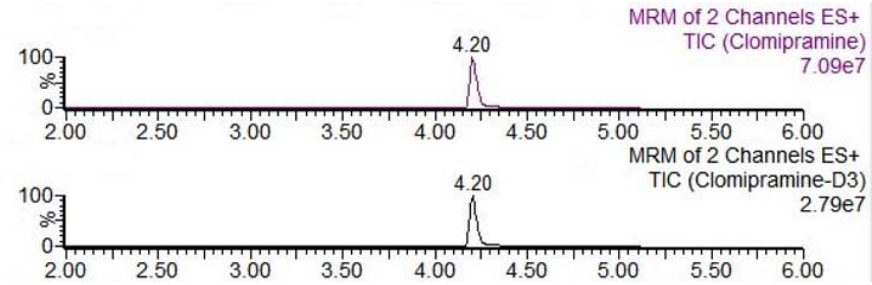


Fig. 2: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top) and internal standard (bottom) of clomipramine.

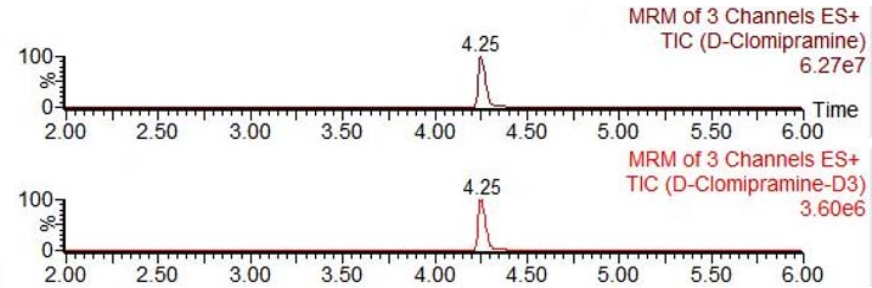


Fig. 3: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top) and internal standard (bottom) of norclomipramine (D-clomipramine).

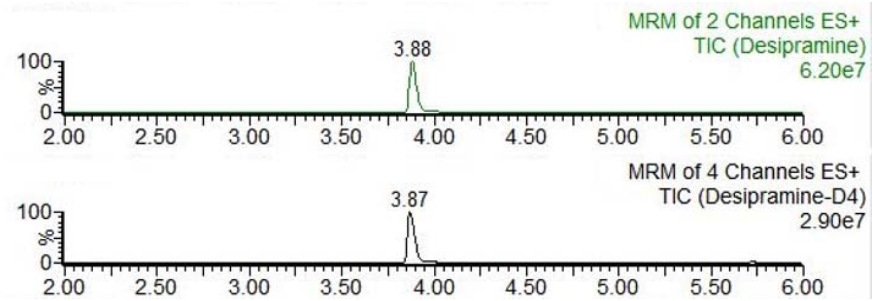


Fig. 4: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top) and internal standard (bottom) of desipramine.

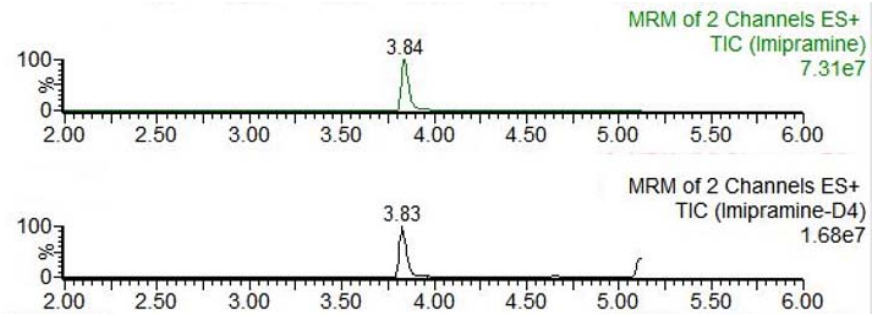


Fig. 5: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top) and internal standard (bottom) of imipramine.

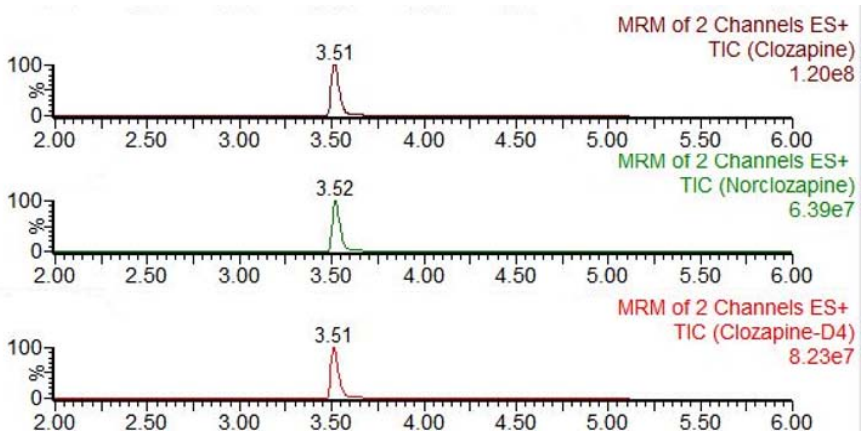


Fig. 6: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top, middle) of clozapine und norclozapine and internal standard (bottom) of clozapine.

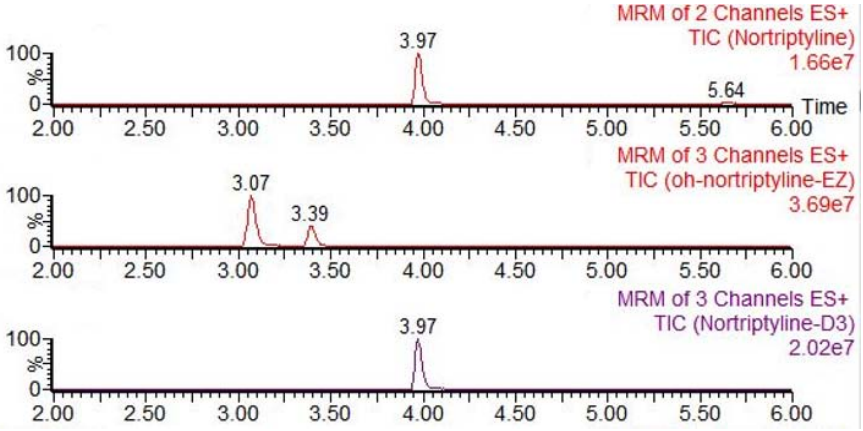


Fig. 7: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top, middle) of nortriptyline und E/Z-10-OH-nortriptyline (E-10-OH-nortriptyline has the shorter retention time) and internal standard (bottom) of nortriptyline.

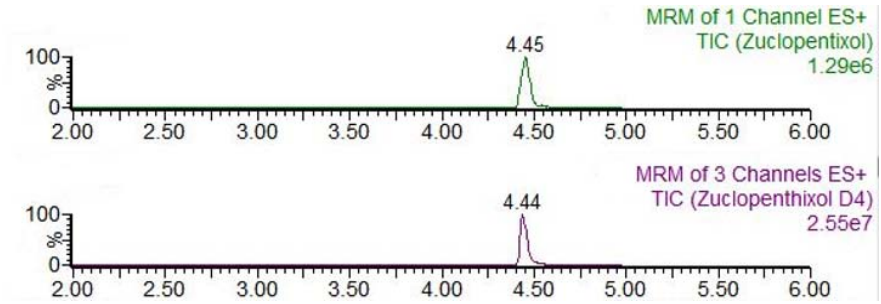


Fig. 8: Chromatogram (recorded with Waters TQS LC-MS/MS) for the analyte (top) and internal standard (bottom) of zuclopenthixol.

## 9. QUALITY CONTROL

Control samples should be analysed with each run. The results of the control samples are used to confirm the accuracy of the method. The test results may not be valid, if one or more values of the quality control sample are outside the acceptable range (see product specification).

### *Results from LC-MS/MS and reference values*

The test gives a specific value for the measurand that must be compared to appropriate reference values to interpret it for the particular patient.

Since the substances measured do not occur naturally in the human body, no classical reference values can be used as with endogenous substances. For pharmaceutical substances, different values apply, such as regular dosage, upper limit and toxicity. These information are (patient-)specific and of technical nature, Immundiagnostik AG refers to the healthcare professional under whose supervision the test is performed.

For interpretation of results, always consult a trained medical professional with expertise in the area of interest for this kit.

Interpretation of the results of this test also depends significantly on the individual characteristics of the patient involved. Immundiagnostik AG recommends taking these into consideration as well.

## 10. TESTCHARACTERISTICS

*Repeatability - intra-day precision*

| | CV | | |
|---|---|---|---|
| **Sample** | **Control 1 [%]** | **Control 3 [%]** | **Patient [%]** |
| Amitriptyline | 3.7 | 1.8 | 2.3 |
| Clomipramine | 2.0 | 1.2 | 1.7 |
| Clozapine | 2.4 | 1.3 | 1.6 |
| Desipramine | 2.8 | 1.5 | 2.2 |
| Imipramine | 4.1 | 2.2 | 3.3 |
| Norclomipramine | 2.3 | 1.2 | 1.8 |
| Norclozapine | 3.3 | 1.9 | 2.2 |
| Nortriptyline | 6.6 | 4.6 | 4.5 |
| E-10-OH-Nortriptyline | 4.0 | 3.5 | 3.5 |
| Z-10-OH-Nortriptyline | 4.1 | 3.5 | 5.3 |
| Zuclopenthixol | 10.6 | 6.0 | 5.8 |

*Reproducibility - inter-day precision*

| | CV | | |
|---|---|---|---|
| **Sample** | **Control 1 [%]** | **Control 3 [%]** | **Patient [%]** |
| Amitriptyline | 8.0 | 5.0 | 4.7 |
| Clomipramine | 6.4 | 4.6 | 5.9 |
| Clozapine | 4.9 | 4.8 | 4.7 |
| Desipramine | 4.9 | 4.4 | 5.5 |
| Imipramine | 7.1 | 6.6 | 6.0 |
| Norclomipramine | 6.8 | 4.9 | 5.5 |
| Norclozapine | 5.0 | 4.4 | 4.7 |
| Nortriptyline | 10.6 | 4.6 | 5.4 |
| E-10-OH-Nortriptyline | 8.1 | 5.0 | 6.0 |
| Z-10-OH-Nortriptyline | 8.1 | 5.1 | 6.1 |
| Zuclopenthixol | 13.0 | 6.8 | 9.1 |

*Measuring range with limit of quantification (LOQ)*

| Analyte | [µg/l] |
|---|---|
| Amitriptyline | < 3.66–1 800 |
| Clomipramine | < 6.12–1 400 |
| Clozapine | < 11.05–2 200 |
| Desipramine | < 4.27–1 200 |
| Imipramine | < 4.97–2 000 |
| Norclomipramine | < 4.31–1 175 |
| Norclozapine | < 6.46–2 600 |
| Nortriptyline | 5.60–1 250 |
| E-10-OH-Nortriptyline | 15.75–1 500 |
| Z-10-OH-Nortriptyline | 4.32–288 |
| Zuclopenthixol | TBD–290 |

## 11. PRECAUTIONS

- Human material used in the kit components was tested and found to be negative for HBsAg and antibody to HIV-1, HIV-2 and HIV p24 Ag, anti-HTLV 1&2 and to HCV and HIV genome as well as syphilis. However, for safety reasons, all kit components should be treated as potentially infectious.
- The GHS symbols indicated on the individual components and specifications of the material safety data sheets (available on request from Immundiagnostik AG) must be noted. When working with these reagents, the legal protective precautions must be adhered to.
- The test components contain organic solvents. Contact with skin or mucous membranes has to be avoided.

## 12. DISPOSAL

Autosampler wash solution (AUTOWASH), mobile phase A (MOPHAA), mobile phase B (MOPHAB) and precipitation reagent (PREC) must be disposed as non-halogenated solvents.

Calibrators (CAL1–6) and controls (CTRL1–3) should be disposed as potentially infectious material in accordance with local regulations.

## 13. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- Plugs and caps of different reagents should not be swapped.
- The individual components of the kit are designed for a maximum of the specified number of test runs. Any part of the components that has already been used must not be reused, but must be disposed of properly in accordance with local regulations.

## 14. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- *IDK*® is a trademark of Immundiagnostik AG.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- Please contact Immundiagnostik AG if one or more components of the kit are damaged, missing (see material supplied) or precipitates are visible in the ready-to-use solutions.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG together with a written complaint.

## 15. REFERENCES


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***Used symbols:***