Labordiagnostische Möglichkeiten zum Nachweis eines (Straßen-) Heroinkonsums bei Patienten in Substitutionstherapie

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LABOR®

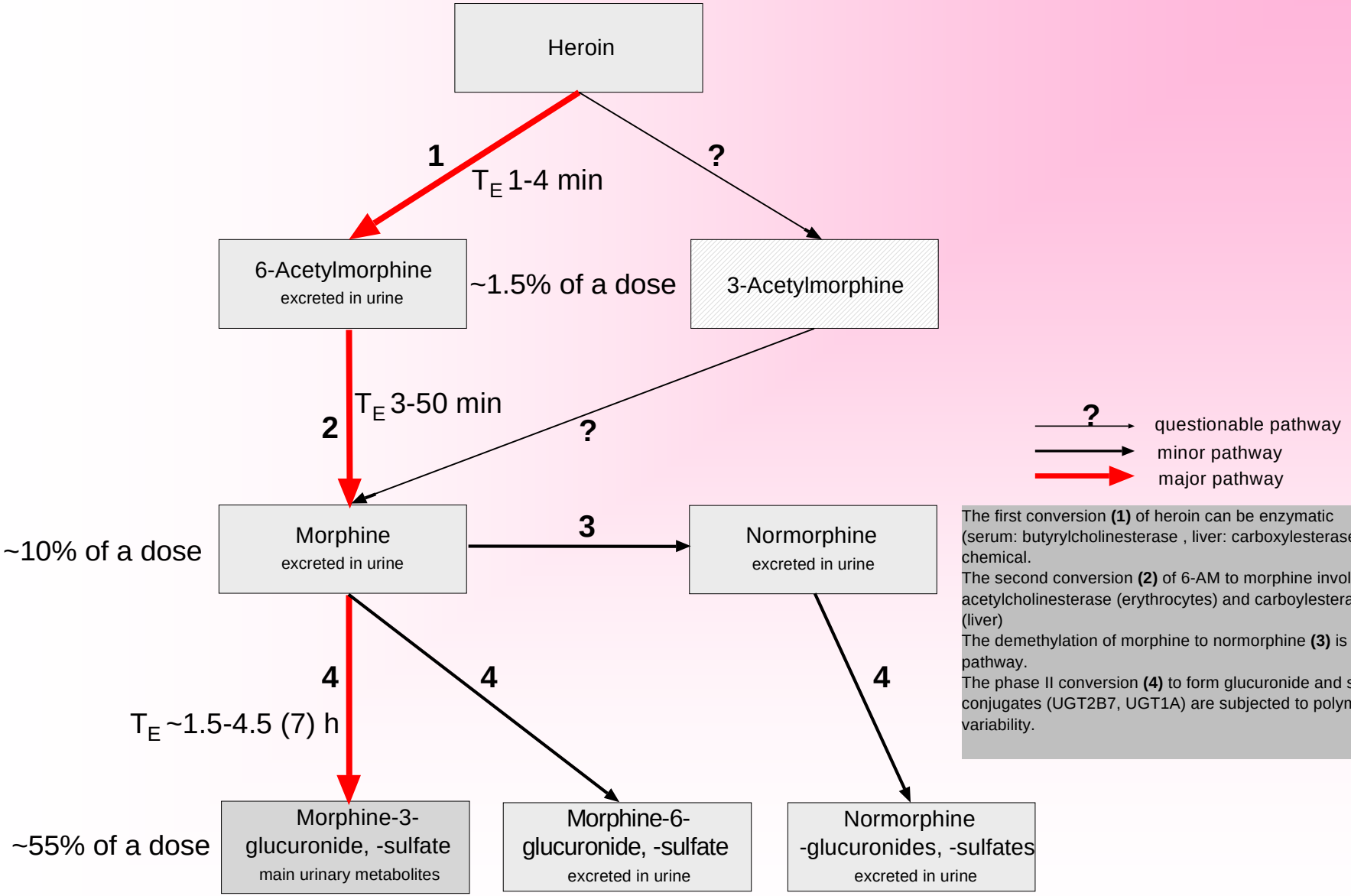
LIMBACH GRUPPE

What is Heroin ?

Heroin = Diacetylmorphine
penetrates blood-brain barrier ~25x faster than Morphine

clandestine/illegal labs derivatize Morphine (acetylation) from Opium

Schematic presentation of the heroin metabolism



The first conversion **(1)** of heroin can be enzymatic (serum: butyrylcholinesterase , liver: carboxylesterases) and chemical.
The second conversion **(2)** of 6-AM to morphine involves acetylcholinesterase (erythrocytes) and carboylesterases (liver)
The demethylation of morphine to normorphine **(3)** is a minor pathway.
The phase II conversion **(4)** to form glucuronide and sulfate conjugates (UGT2B7, UGT1A) are subjected to polymorphic variability.

What is Heroin ?

Heroin = Diacetylmorphine
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Opium:
Scratching the fruit of the of the poppy plant Papaver somniferum
the dried latex juice ("poppy tears") is then scraped off

Opium contains several alkaloids:
<0.1 - 450 µg/g Morphine
<0.1 - 57 µg/g Codeine
0.3 - 41 µg/g Thebaine
0.84 - 230 µg/g Noscapine
0.0 - 67 µg/g Papaverine

The latex juice contaminates the poppy seeds! Washing of the seeds is decisive…….

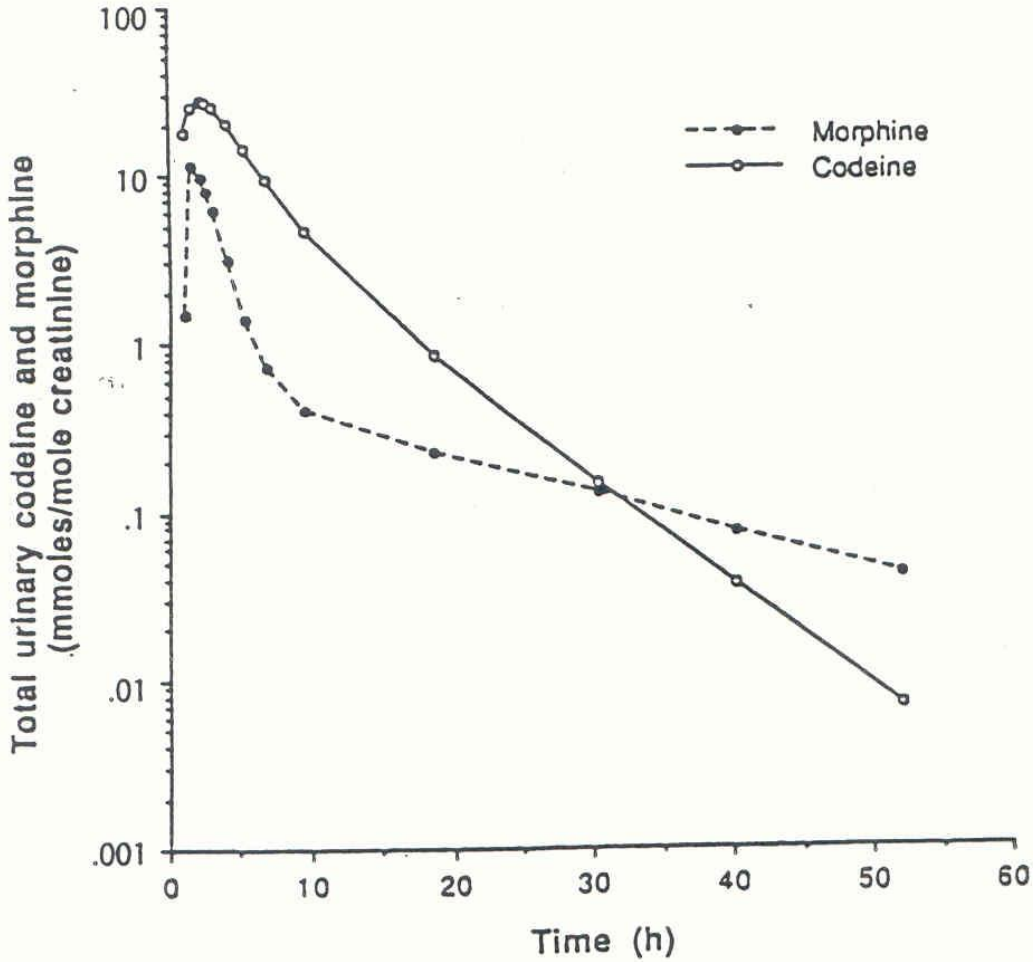
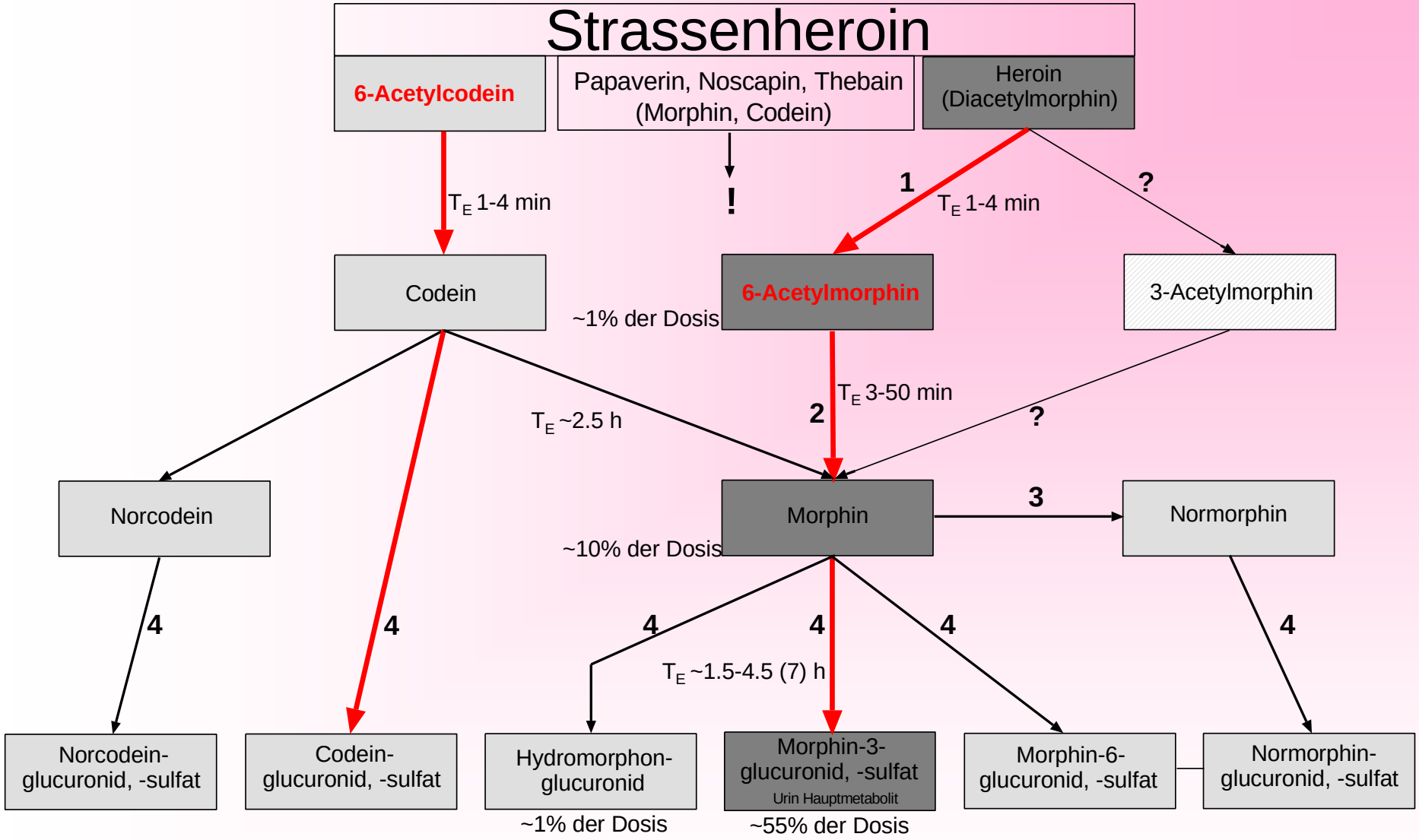


Figure 1. The urinary excretion of total codeine and morphine after single dose intake of 50 mg codeine orally by 13 healthy volunteers. The graph was constructed by fitting individual data to a two-compartment model (three exponentials, weighting factor $1/y^2$).

Lin et al.,
JAT 18, 1994



Cave! Substitol enthält <0.1% Codein

6-AM study urine

Study period:	~11 weeks, 49 consecutive workdays, all spls. tested for 6-AM (CEDIA) and Opiates (CEDIA), Hit 911. Confirmation with GC/MS-SIM after SPE and PFP acylation LoD 6-AM: 0.5 ng/mL
Patients:	**707 patients**, 486 men (69%) + 221 women (31%)
Samples:	**3521 samples** from 6 different outpatient clinics of different size in Berlin
Substitutes:	Methadone (581 patients = 82%) L-Polamidon® (= l-methadone ; 67 patients = 9%) Subutex® (= buprenorphine ; 59 patients = 8%)
Dosing ranges:	methadone: 1-300 mg/d l-methadone: 5-140 mg/d buprenorphine: 0.2-24.0 mg/d
6-AM CEDIA:	cal.-points: 0, 5, 10, 20 ng/mL ; cutoffs: 5, 10 ng/mL
Opiates CEDIA:	cal.-points: 0, 100, 300, 800, 2000 ng/mL ; cutoffs: 100, 300, 2000 ng/mL

Frequency of positive results in the screening for Opiates and 6-AM

	No. of samples	% of total (n = 3521)	No. of patients	% of total (n = 707)
CEDIA opiates				
>= 100 ng/mL	845	**24.0**	325	46.0
>= 300 ng/mL	797	22.6	306	43.3
>= 2000 ng/mL	649	18.4	258	36.5
CEDIA 6-AM				
>= 5 ng/mL	370	**10.5**	192	27.2
>= 10 ng/mL	319	9.1	169	23.9
>= 20 ng/mL	269	7.6	149	21.1

44% of Opiates positives

Confirmatory analysis performed with GC/MS

Correspondence of 6-AM and Opiates CEDIA semiquant. results

6-AM response [ng/mL]	**No. of spls. with opiate response <100 ng/mL** (% of total)	**No. of spls. with opiate response <300 ng/mL** (% of total)	**No. of spls. with opiate response <2000 ng/mL** (% of total)	**No. of spls. with opiate response >=2000 ng/mL** (% of total)	**total no. of spls.**
>= 20	6 (2.2)	17 (6.3)	20 (7.4)	249 (92.6)	269
>= 10	10 (3.1)	23 (7.2)	27 (8.5)	292 (91.5)	319
>= 5	14 (3.8)	28 (7.6)	33 (8.9)	337 (91.1)	370

Journal of Analytical Toxicology, Vol. 30, March 2006

Paradoxical Results in Urine Drug Testing for 6-Acetylmorphine and Total Opiates: Implications for Best Analytical Strategy

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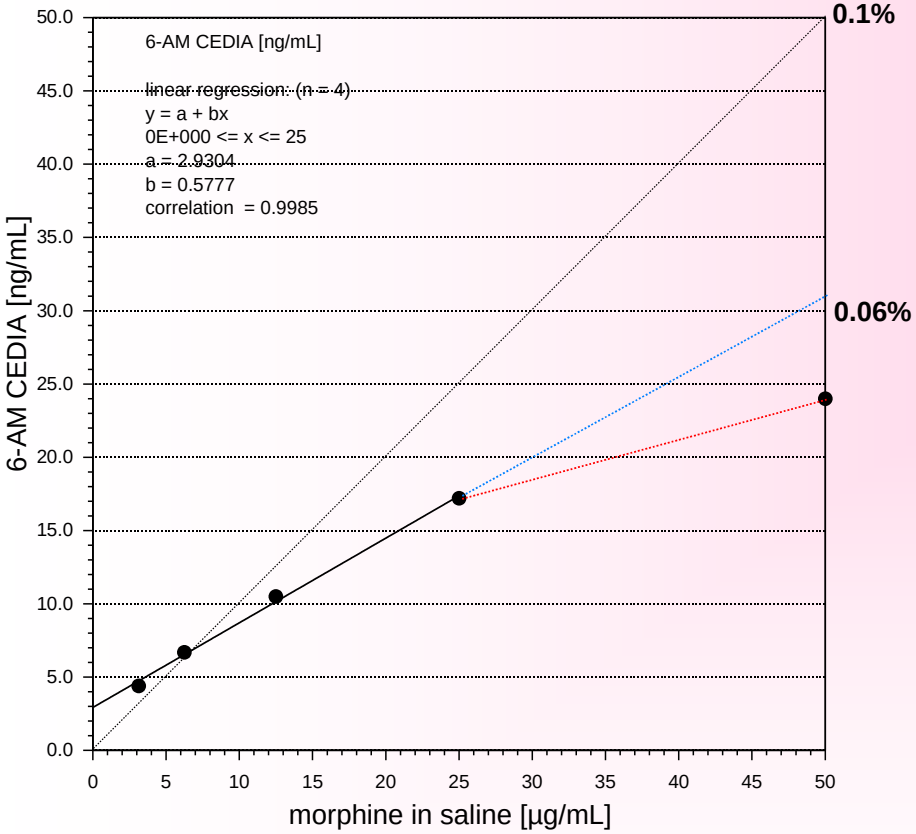
Instrument-based immunoassays

CEDIA: unwanted X-reactivity

Free morphine in the 6-AM CEDIA



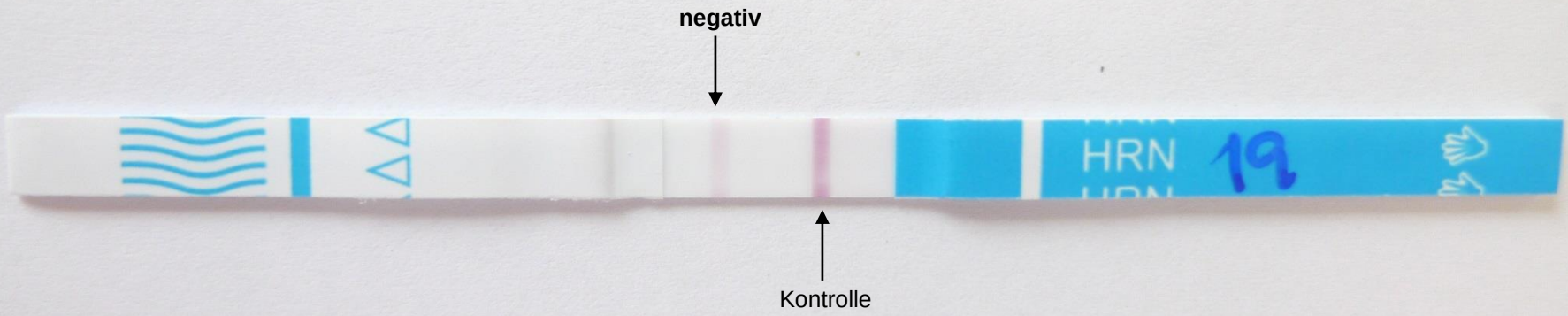
J Anal Toxicol. 2005 Apr;29(3):201-4.
Evaluation of the CEDIA heroin metabolite (6-AM) immunoassay with urine specimens from a criminal justice drug-testing program.
Jenkins AJ1, Lavins ES, Snyder A.

Siemens 6-AM EMIT

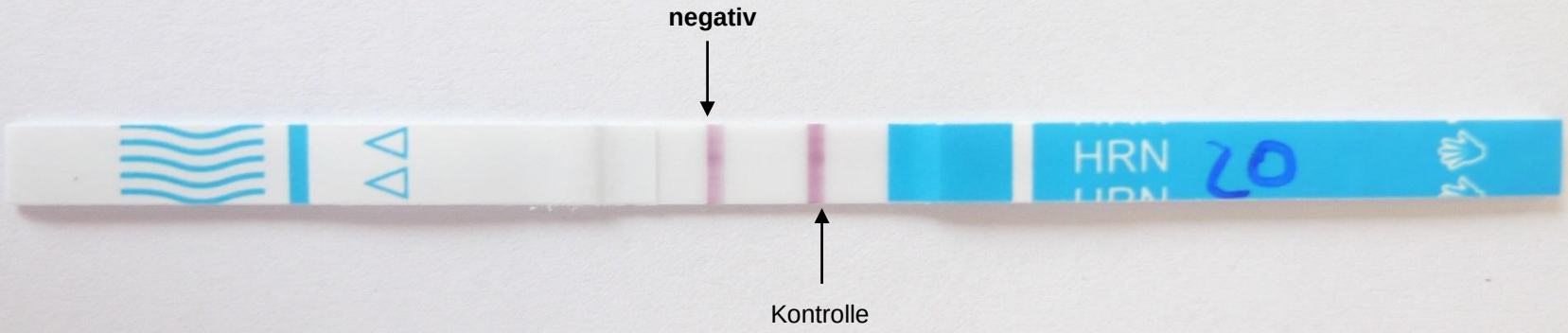
Assay Specificity
Concentrations of compounds that produce negative results vs. the 10 ng/mL cut-off

Compounds Concentration Tested	(ng/mL)
Buprenorphine	1,000,000
Codeine	500,000
Dextromethorphan	100,000
Dihydrocodeine	500,000
Heroin	80
Hydrocodone	300,000
Hydromorphone	100,000
Imipramine	200,000
Levorphanol	100,000
Meperidine	800,000
Morphine	100,000
Morphine-3-Glucuronide	600,000
Morphine-6-Glucuronide	600,000
Nalorphine	100,000
Naloxone	300,000
Naltrexone	300,000
Norcodeine	600,000
Normophine	100,000
Oxycodone	400,000
Oxymorphone	100,000

Lfm-diagnostika
Austestung 6-MAM Schnelltest, Cutoff 10 ng/mL
NaCl 0.9%

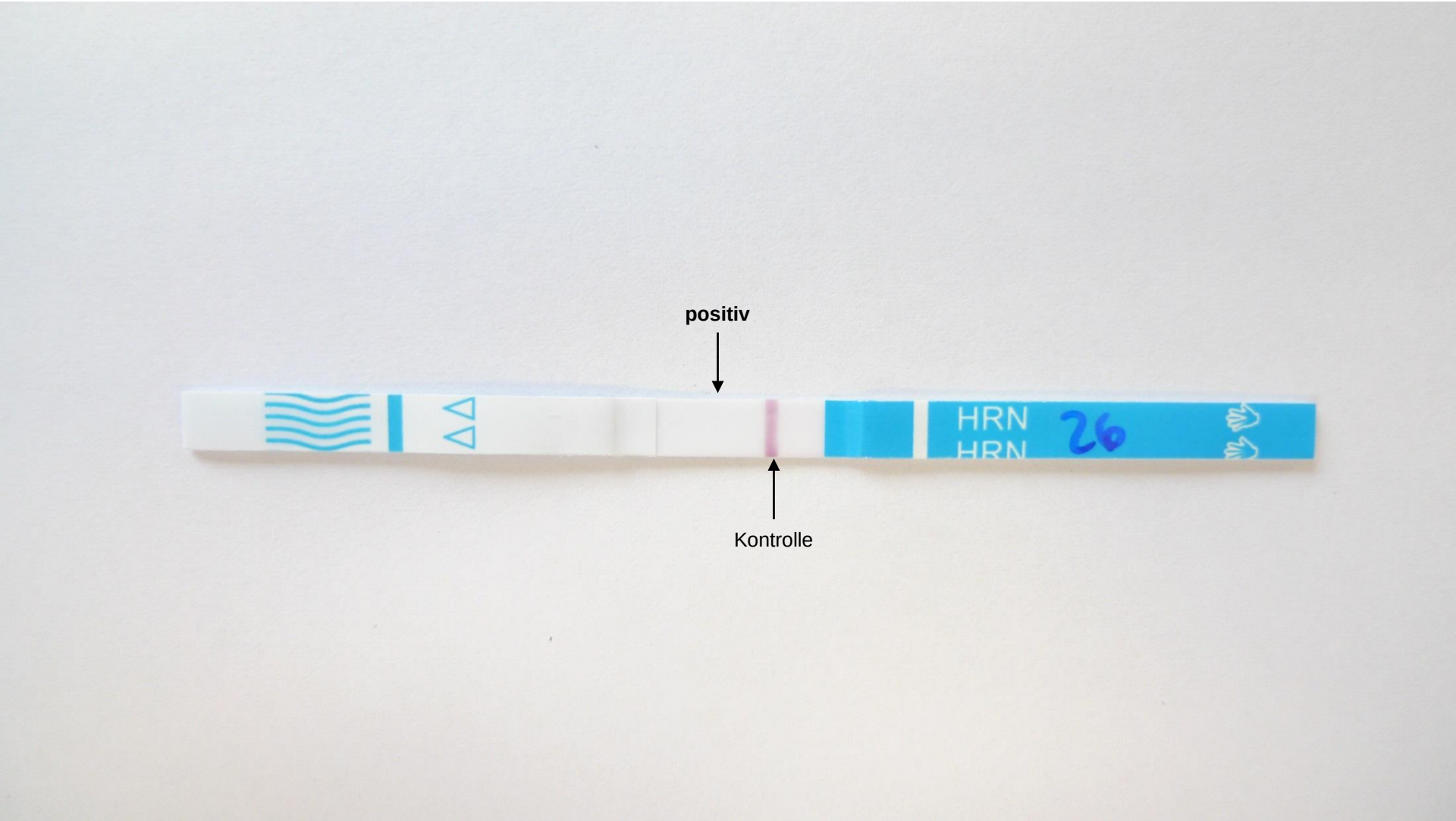


Austestung 6-MAM Schnelltest
realer negativ Urin, Kreatinin 120 mg/dL

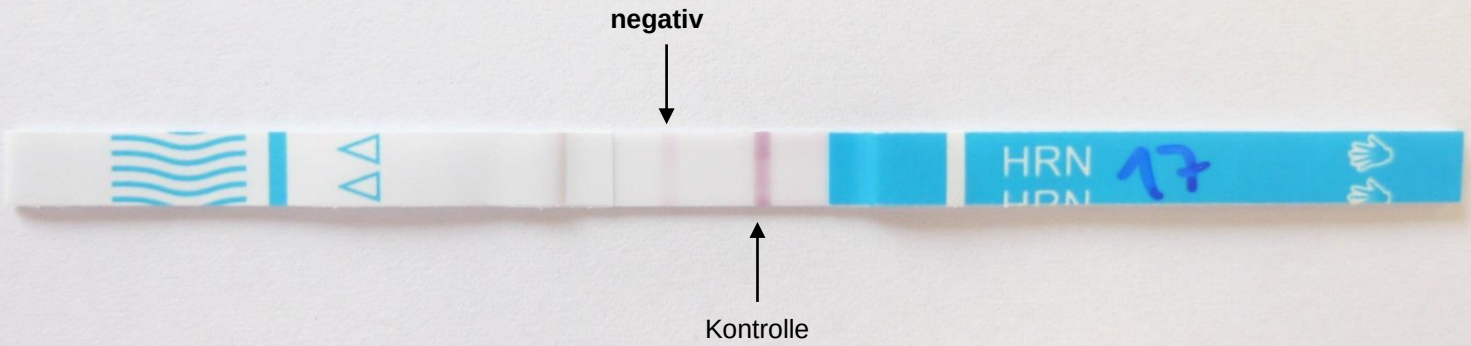


Austestung 6-MAM Schnelltest

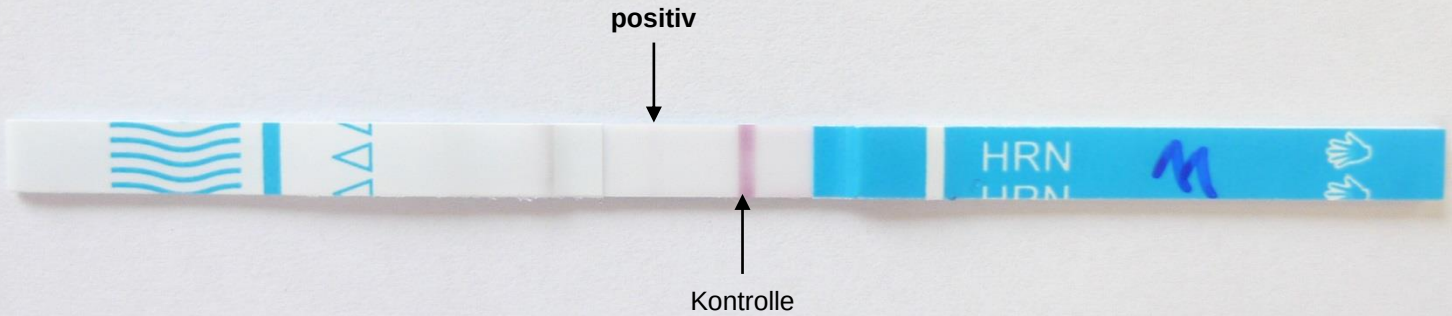
6-Acetylmorphin in NaCl 0.9%, -25% Cutoff, 7.5 ng/mL



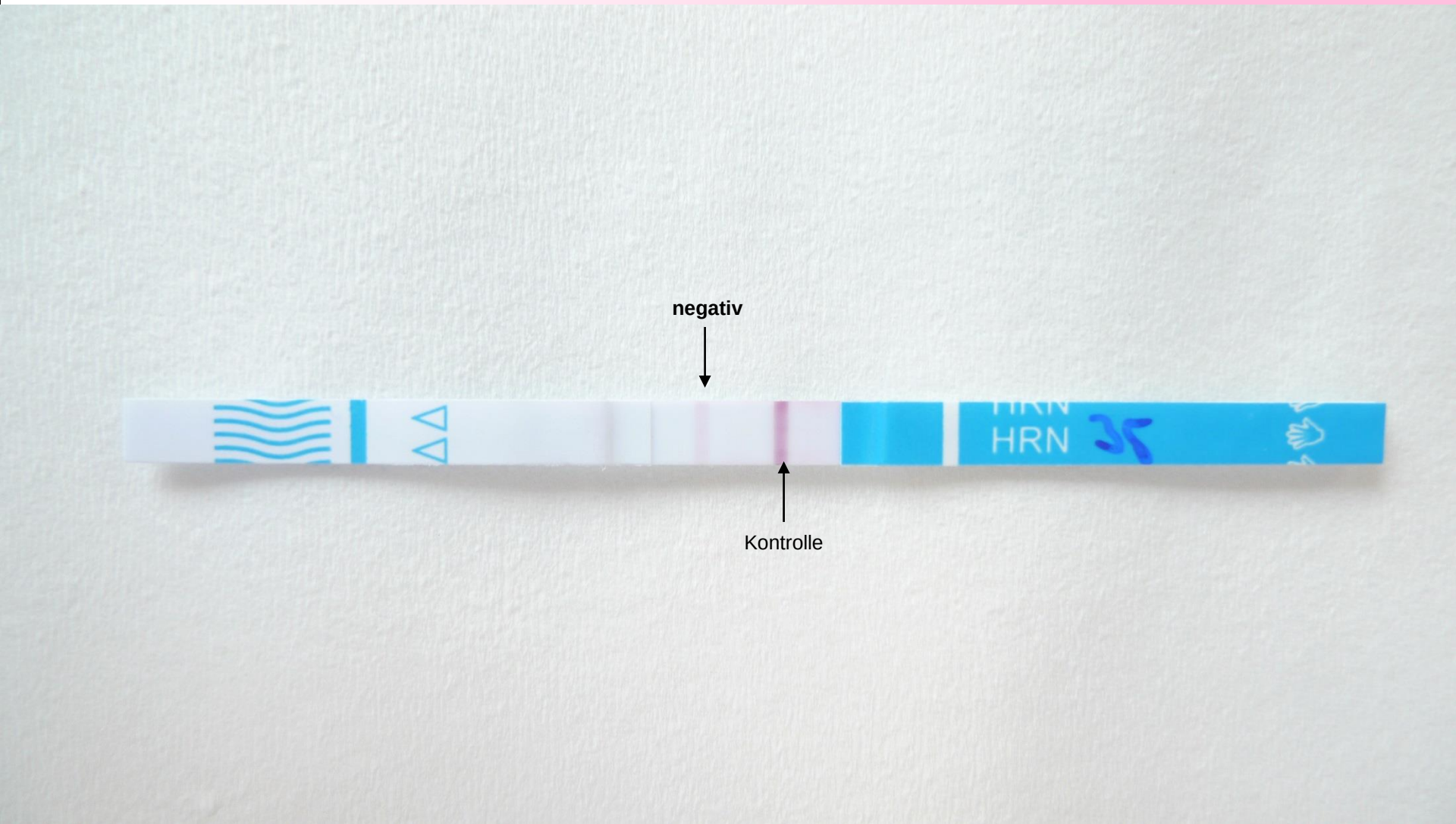
Austestung 6-MAM Schnelltest
Codein in NaCl 0.9%, 100 µg/mL



Austestung 6-MAM Schnelltest
Codein-6-Glucuronid in NaCl 0.9%, 100 µg/mL



Austestung 6-MAM Schnelltest
Codein-6-Glucuronid in NaCl 0.9%, 10 µg/mL



Analyt	Konzentration [µg/mL]	Teststreifen 6-AM po/swpo/ne
Methadon	100	ne
EDDP	100	ne
Buprenorphin	100	ne
Buprenorphin-Glucuronid	100	ne
Buprenorphin-Glucuronid	10	ne
Norbuprenorphin	100	po
Norbuprenorphin-Glucuronid	100	ne
Norbuprenorphin-Glucuronid	10	ne
Dihydrocodein	100	swpo
Dihydrocodein-6-Glucuronid	100	swpo
Diacetylmorphin	100	po
Morphin	100	po
Morphin	10	ne
Morphin-3-Glucuronid	100	ne
Morphin-6-Glucuronid	100	ne
3-Acetylmorphin	100	po
6-Acetylmorphin	100	po
Codein	100	ne
Codein-6-Glucuronid	100	po
Codein-6-Glucuronid	10	ne
6-Acetylcodein	100	po
6-Acetylcodein	10	po
Naltrexon	100	swpo
6ß-Naltrexol	100	ne
6ß-Naltrexol	10	ne
Naloxon	100	swpo
Oxymorphon	100	po
Oxycodon	100	po
Hydromorphon	100	po

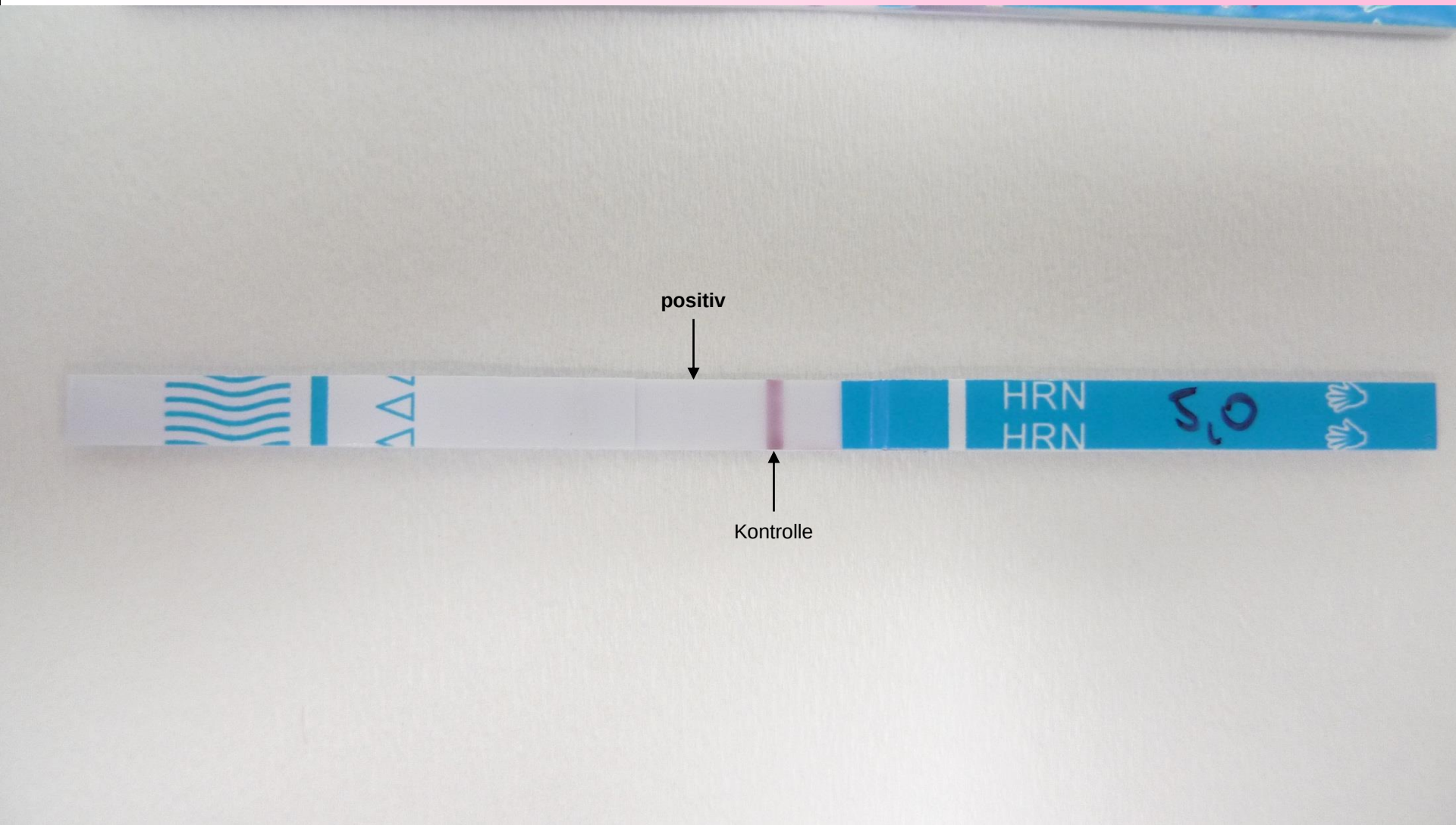
Analyt	Konzentration [µg/mL]	Teststreifen 6-AM po/swpo/ne
6-Acetylmorphin -25% Cutoff (10 ng/mL)	7.5 ng/mL	po
6-Acetylmorphin +25% Cutoff (10 ng/mL)	12.5 ng/mL	po
6-Acetylmorphin Specialty HEIA Ktrl. 7.5	7.5 ng/mL	po
6-Acetylmorphin Specialty HEIA Ktrl. 12.5	12.5 ng/mL	po
6-Acetylmorphin Specialty HEIA Ktrl. 7.5 1:2 verd. mit NaCl 0.9%	3.75 ng/mL	po

6-AM Schnelltest lfm-diagnostika: Überprüfung der **Spezifität**

Sensitivität

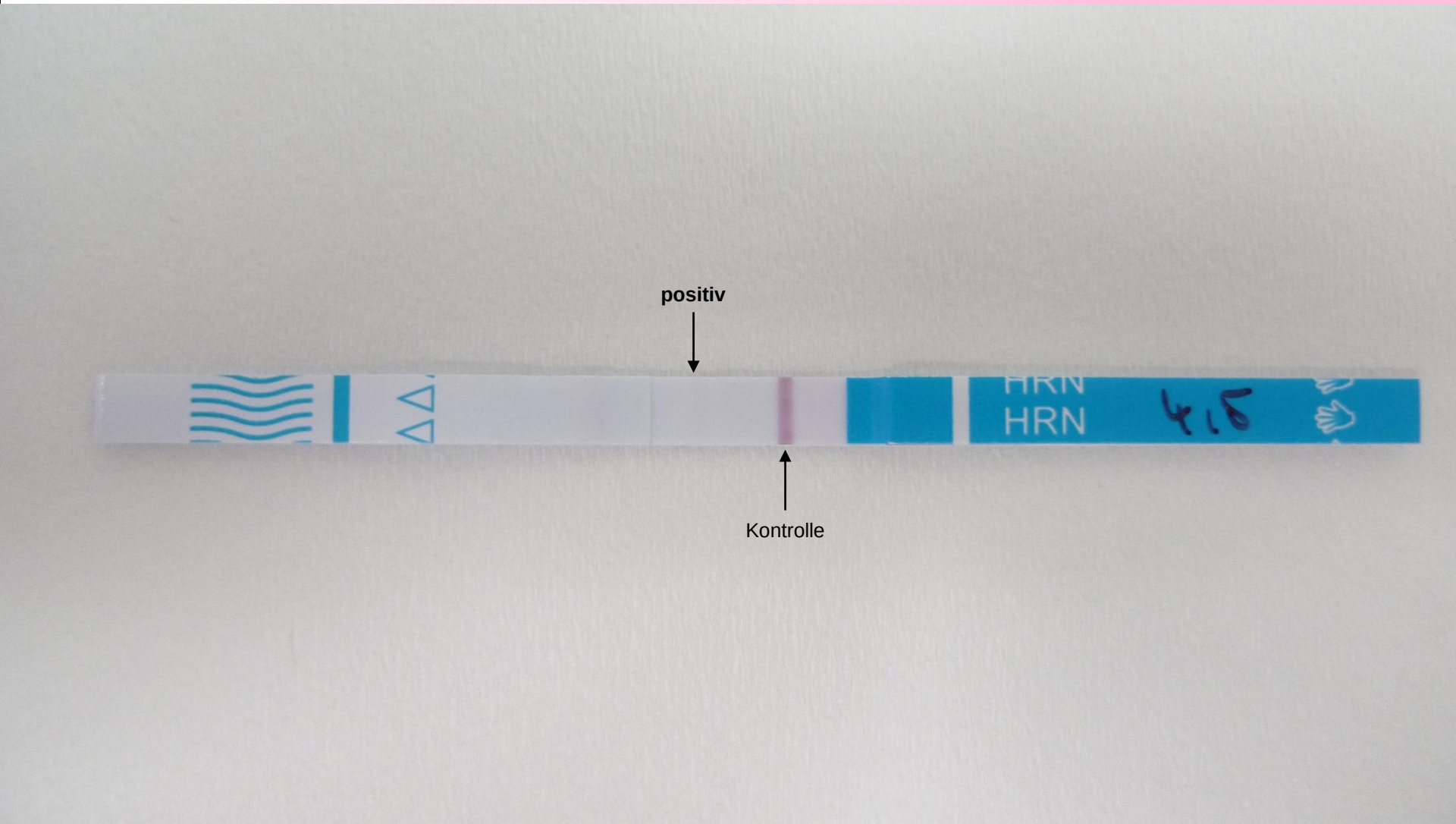
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 5.0 ng/mL



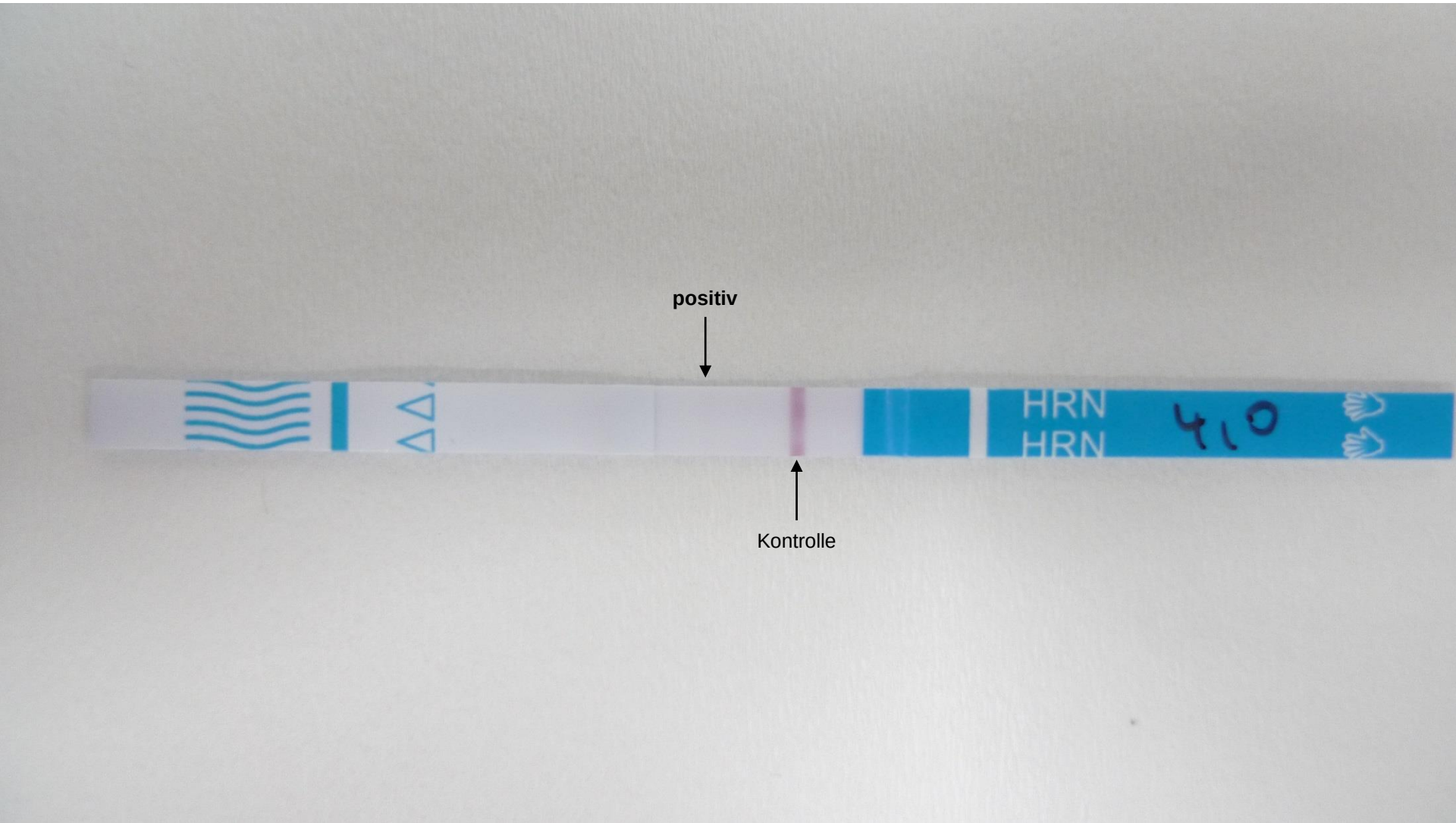
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 4.5 ng/mL



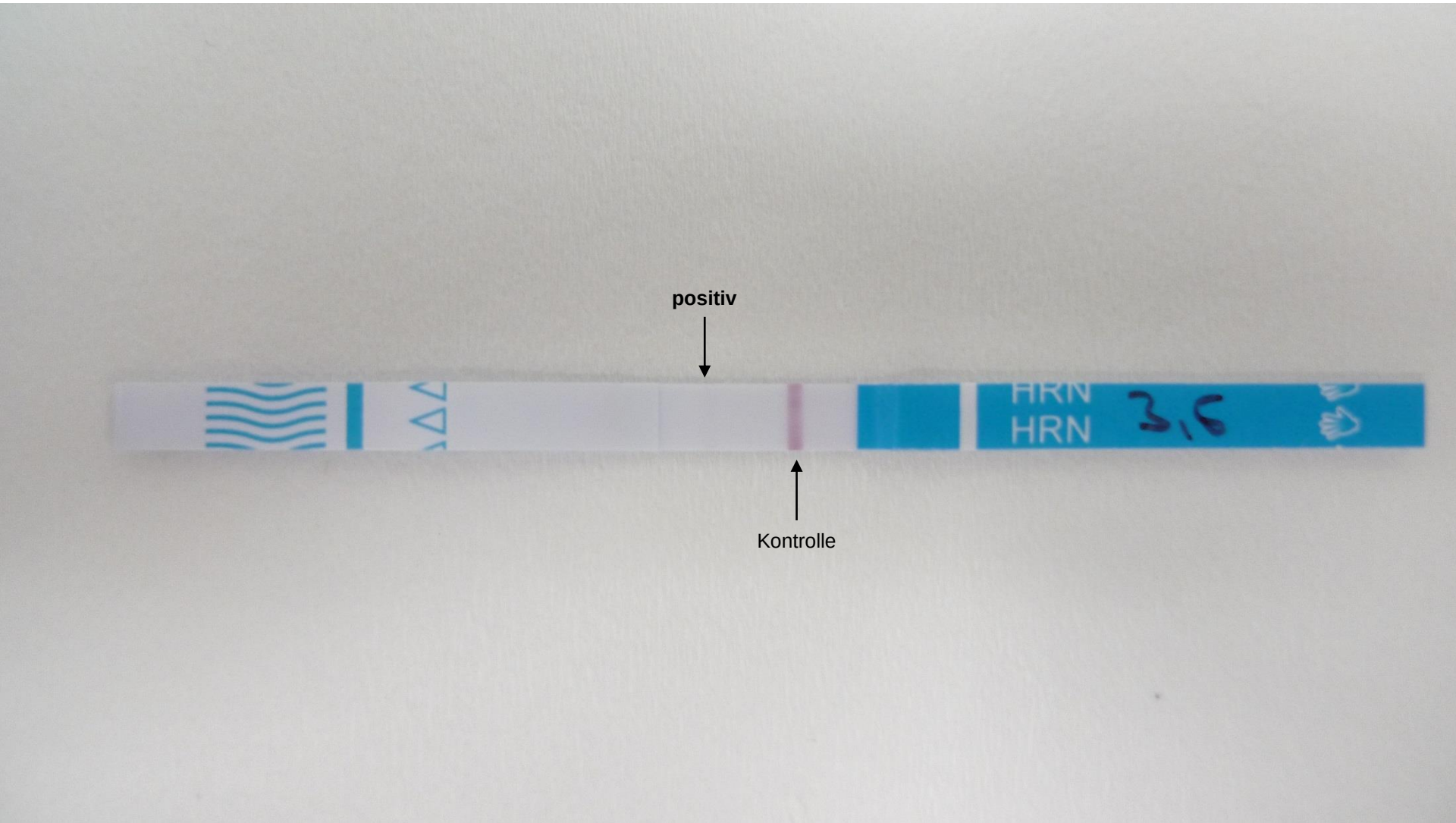
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 4.0 ng/mL



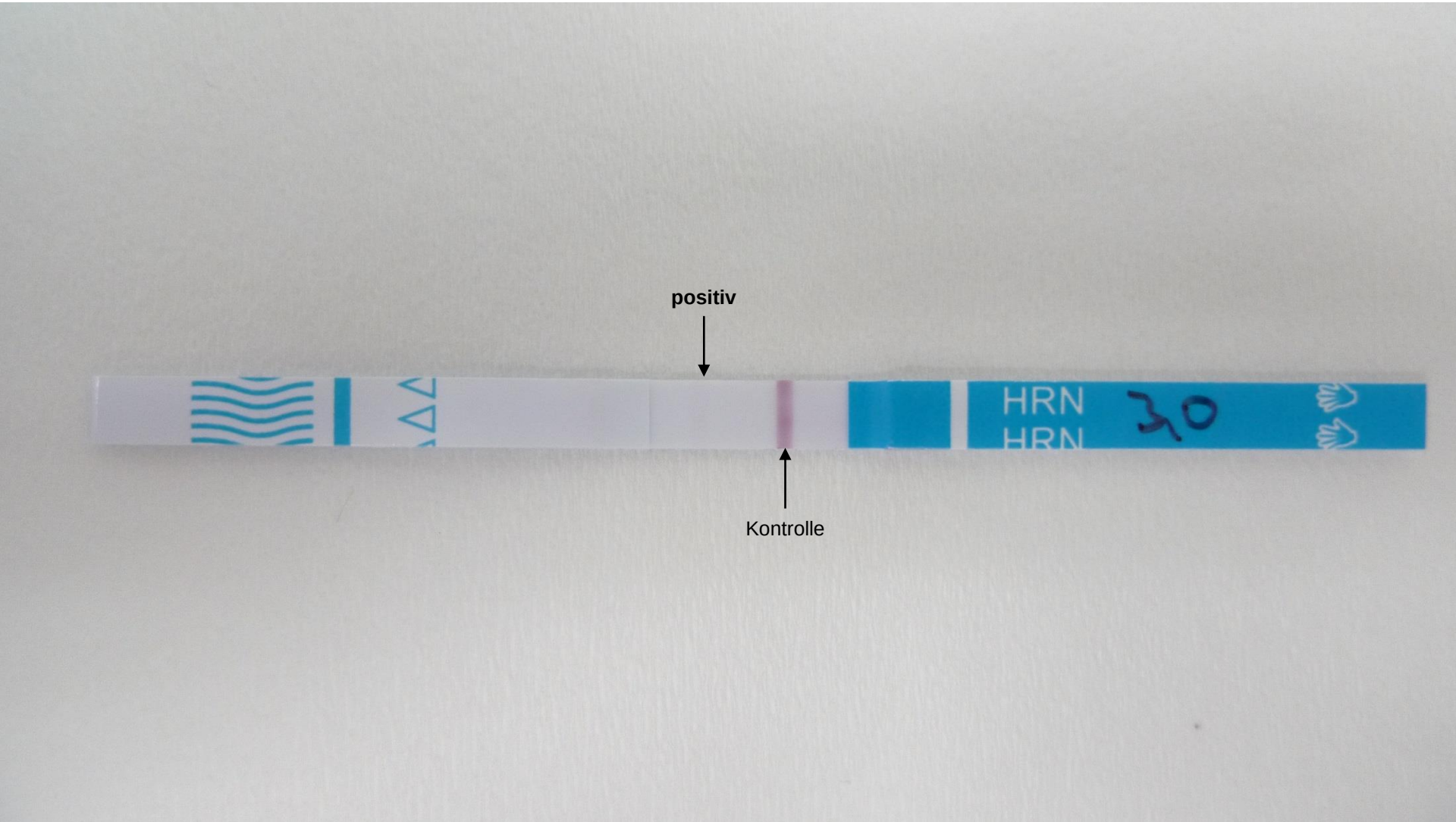
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 3.5 ng/mL



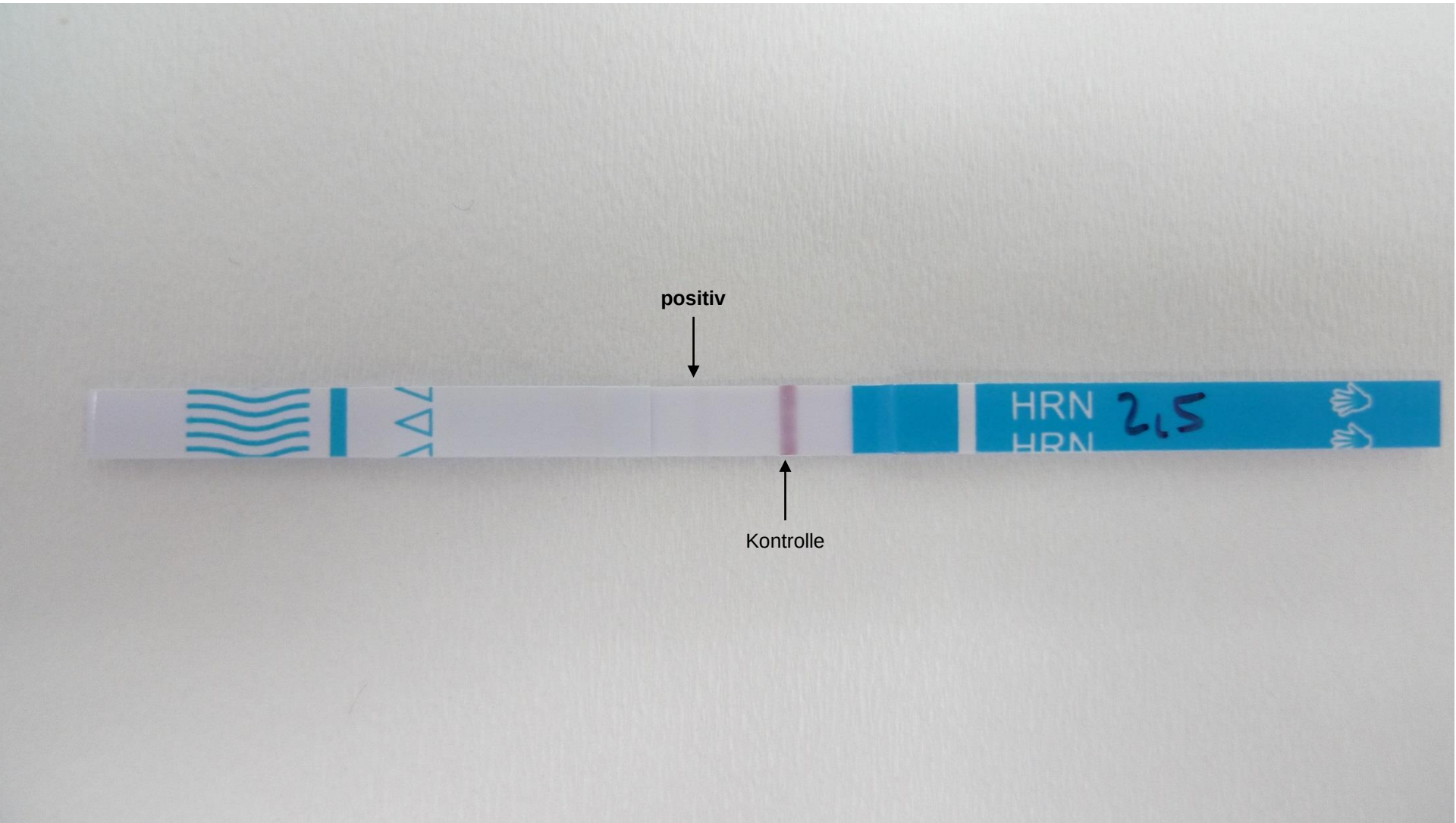
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 3.0 ng/mL



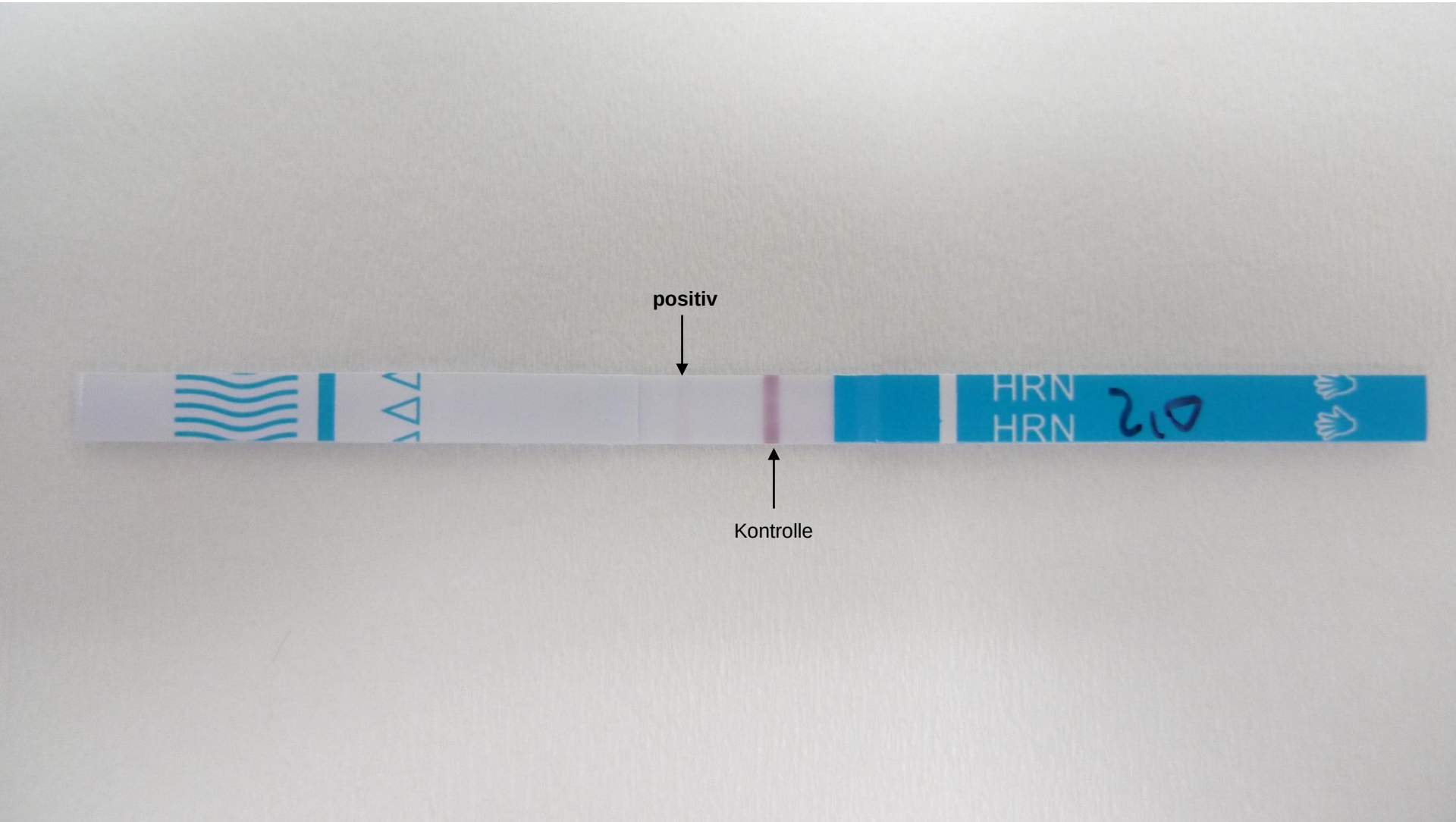
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 2.5 ng/mL



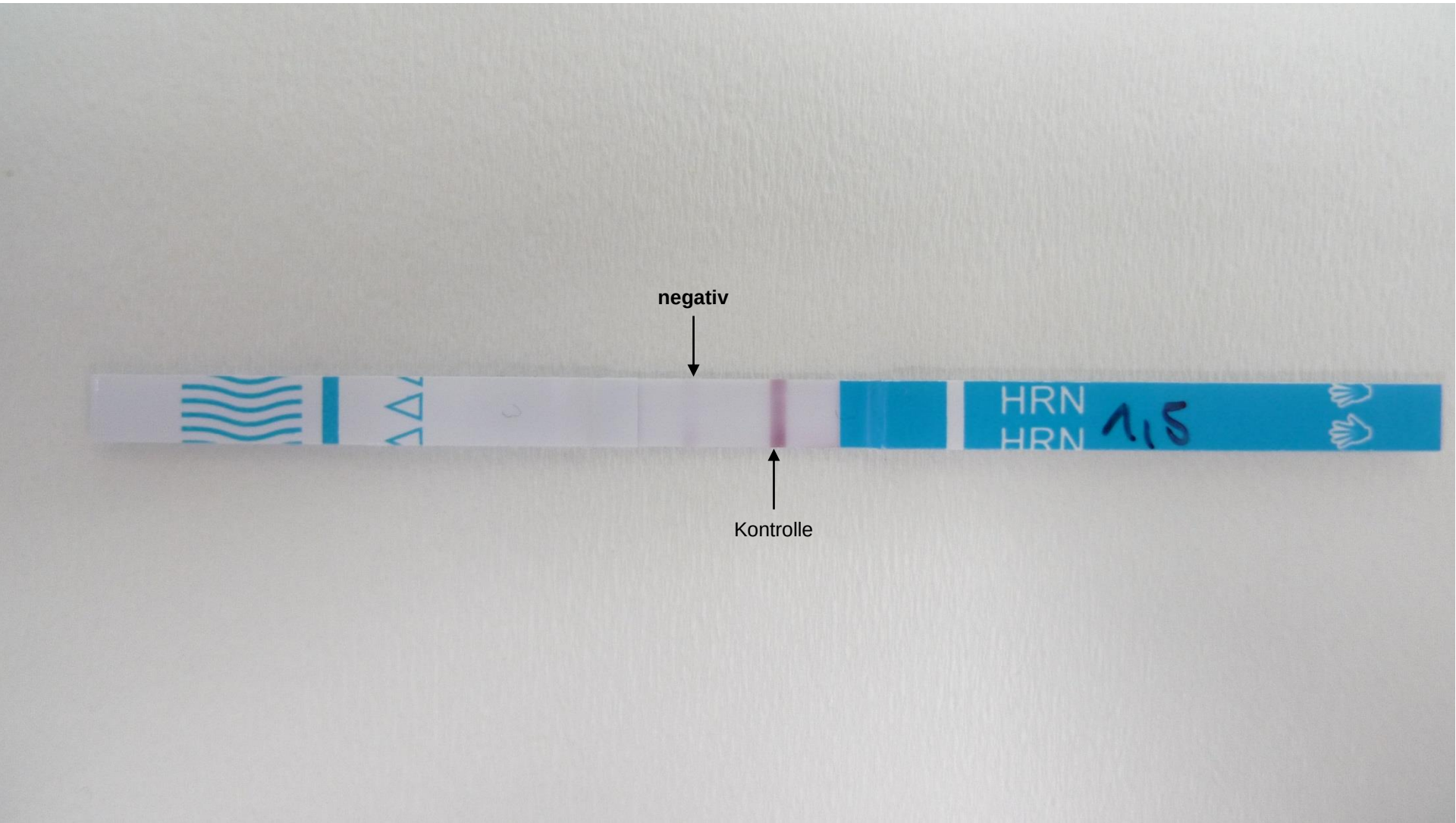
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 2.0 ng/mL



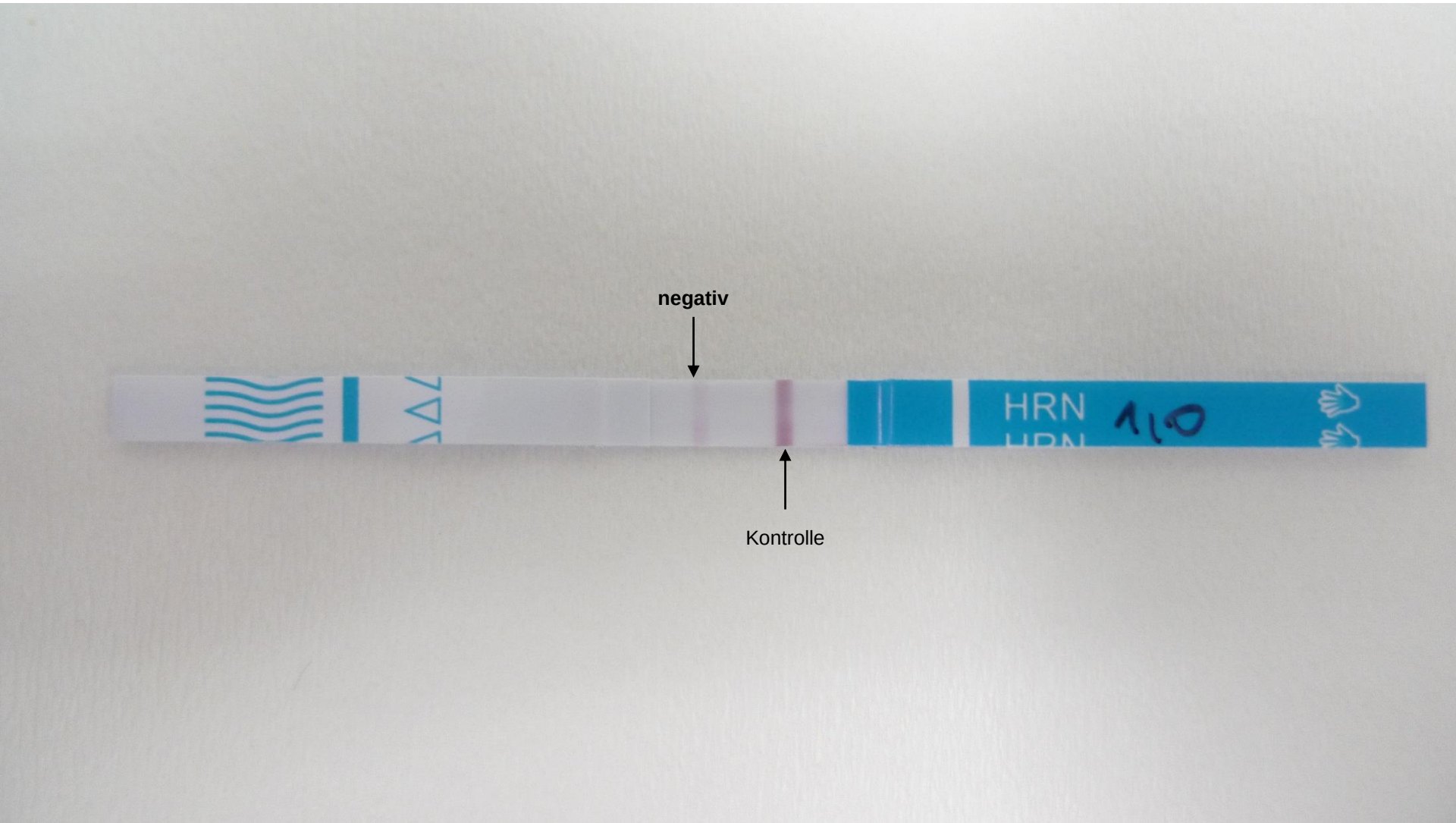
Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 1.5 ng/mL



Austestung 6-MAM Schnelltest

6-Acetylmorphin in NaCl 0.9%, 1.0 ng/mL



Austestung 6-MAM Schnelltest

realer Urin, Kreatinin 16 mg/dL, Substitut: Methadon

Immunchemisches Screening

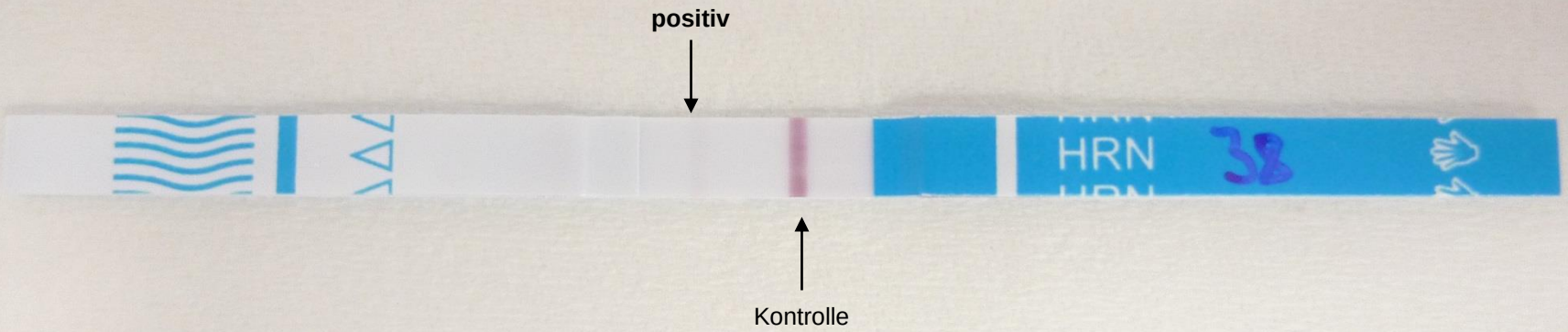
Opiate (DRI, Microgenics):	>2000 ng/mL
6-Acetylmorphin (HEIA, Specialty):	**6.3 ng/mL**

LFM Diagnostika 6-MAM

6-Acetylmorphin:	**positiv**

Multitargetscreening Opiate im Urin mit LC-MS/MS

"freies" Morphin:	226 ng/mL
Morphin-3-Glucuronid:	3619 ng/mL
Morphin-6-Glucuronid:	578 ng/mL
6-Acetylmorphin:	**3.2 ng/mL**
"freies" Codein:	22 ng/mL
Codein-6-Glucuronid:	294 ng/mL
"freies" Norcodein:	16 ng/mL
6-Acetylcodein:	<1.0 ng/mL
"freies" Hydromorphon:	5.0 ng/mL
Hydromorphon-Glucuronid:	nachweisbar
Meconin:	nachweisbar
Papaverin:	negativ
Noscapin:	nachweisbar
Methadon:	1089 ng/mL
EDDP:	16035 ng/mL



Austestung 6-MAM Schnelltest

realer Urin, Kreatinin 189 mg/dL, Substitut: Substitol

Immunchemisches Screening

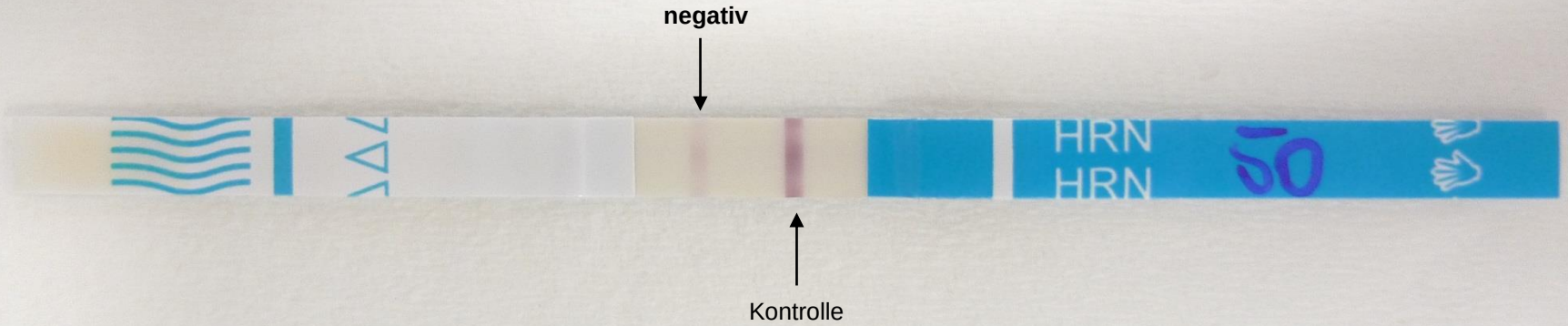
Opiate (DRI, Microgenics):	>2000 ng/mL
6-Acetylmorphin (HEIA, Specialty):	**6.1 ng/mL**

LFM Diagnostika 6-MAM

6-Acetylmorphin:	**negativ**

Multitargetscreening Opiate im Urin mit LC-MS/MS

"freies" Morphin:	96730 ng/mL
Morphin-3-Glucuronid:	352795 ng/mL
Morphin-6-Glucuronid:	196207 ng/mL
6-Acetylmorphin:	**<1.0 ng/mL**
"freies" Codein:	67 ng/mL
Codein-6-Glucuronid:	1551 ng/mL
"freies" Norcodein:	<1.0 ng/mL
6-Acetylcodein:	<1.0 ng/mL
"freies" Hydromorphon:	605 ng/mL
Hydromorphon-Glucuronid:	nachweisbar
Meconin:	negativ
Papaverin:	negativ
Noscapin:	negativ
Methadon:	<1.0 ng/mL
EDDP:	<1.0 ng/mL
Pregabalin:	984738 ng/mL



Methodenvergleich:

Probenanzahl = 53 (Substitol = 30 / Polamidon = 3 / Methadon = 14 / Buprenorphin = 6)

6-AM alle Pat. (n = 53)	LC-MS/MS +	LC-MS/MS -
Teststreifen +	32	0
Teststreifen -	0	21

Konz. Bereich **positiv**:
6-AM: 2 – 13027 ng/mL (mean: 1050 ng/mL / median: 32 ng/mL)

Konz. Bereich 6-AM **negativ**:
Morphin: 541 – 96730 ng/mL
Morphin-3-Glucuronid: 16148 – 361733 ng/mL
Morphin-6-Glucuronid: 4091 – 196207 ng/mL
Codein: 1 – 122 ng/mL (6 = <1.0 ng/mL)
Codein-6-Glucuronid: 7 – 4211 ng/mL
Norcodeine: 5 – 75 ng/mL (13 = <1.0 ng/mL)
Hydromorphon: 2 – 708 ng/mL (1 = <1.0 ng/mL)

6-AM ohne Subst. Pat. (n = 23)	LC-MS/MS +	LC-MS/MS -
Teststreifen +	22	0
Teststreifen -	0	1

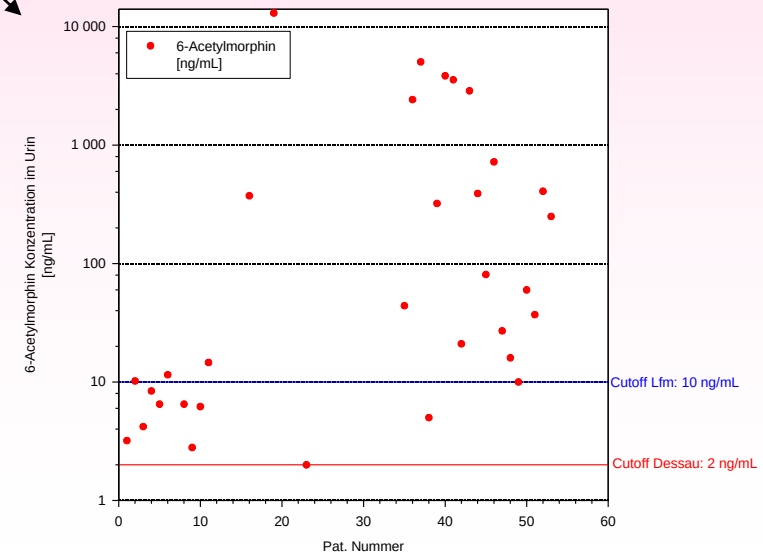
Konz. Bereich **positiv**:
6-AM: 2.8 – 3844 ng/mL

Konz. Bereich 6-AM **negativ**:
Morphin: 1351 ng/mL
Morphin-3-Glucuronid: 33082 ng/mL
Morphin-6-Glucuronid: 7220 ng/mL
Codein: 67 ng/mL
Codein-6-Glucuronid: 4211 ng/mL
Norcodeine: 75 ng/mL
Hydromorphon: 15 ng/mL

6-AM Substitol Pat. (n = 30)	LC-MS/MS +	LC-MS/MS -
Teststreifen +	10	0
Teststreifen -	0	20

Konz. Bereich **positiv**:
6-AM: 2 – 13027 ng/mL

Konz. Bereich 6-AM **negativ**:
Morphin: 541 – 96730 ng/mL
Morphin-3-Glucuronid: 16148 – 361733 ng/mL
Morphin-6-Glucuronid: 4091 – 196207 ng/mL
Codein: 1 – 122 ng/mL (6 = <1.0 ng/mL)
Codein-6-Glucuronid: 7 – 1551 ng/mL
Norcodeine: 5 – 39 ng/mL (13 = <1.0 ng/mL)
Hydromorphon: 2 – 708 ng/mL (1 = <1.0 ng/mL)



Drogenscreening (mit Immunoassay) im Urin -- Probleme:

-- in vivo/vitro Verdünnung! Kreatinin korrigierter Cutoff?!

-- pH-Wert: 4.5 bis 9.0!!

-- Manipulation der Probe: Verdünnung, Detergenzien etc., Oxidantien, Abgabe „Freundurin“, Zugabe d. Medikamente: Probennahme unter Aufsicht

-- Cutoffs: insbes. Gruppenteste nicht standardisiert

-- Kreuzreaktivität: falsch positiv / **falsch negativ**

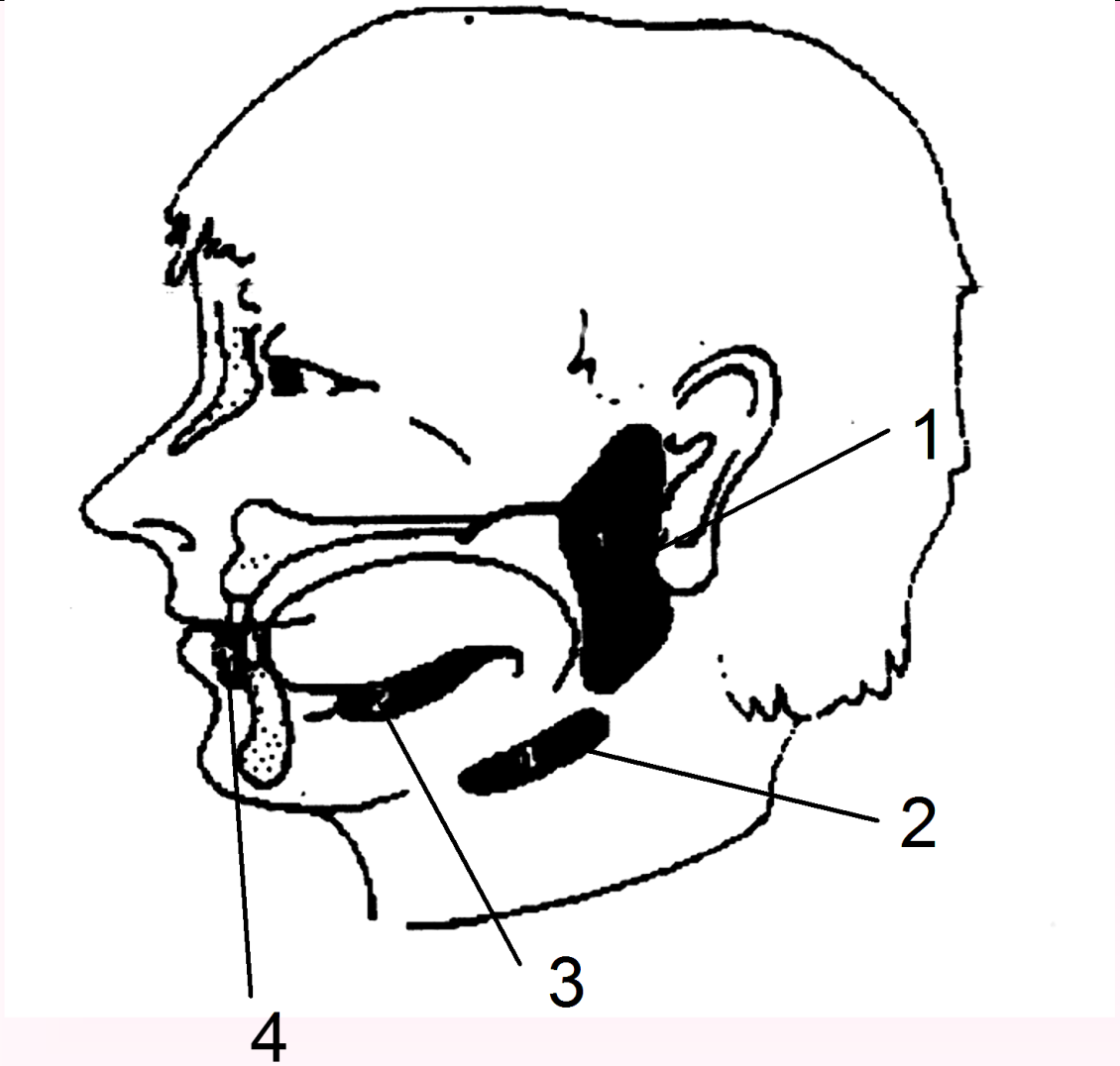
-- Zunehmende Zahl von Drogen, neue **Substanzklassen,** unbekannte Metaboliten

→ **andere Matrix** (Muttersubstanzen)
→ **andere** (sensitivere) **Methoden**

Alternatives Probenmaterial.....

Nicht invasive Probennahmen:

1. **Kapillarblut**
2. **Speichel („Oral Fluid“)**



Bezeichnung der Speicheldrüsen
1 paarige Ohrspeicheldrüse (*Glandula parotis*), 2 Unterkieferdrüse (*Gl.submandibular*), 3 Unterzungendrüse (*Gl.sublingualis*), 4 Lippendrüsen (*Gl.labiales*) [4]

Parotisspeichel: dünnflüssig, nicht fadenziehend
Submandibularisspeichel: klar, schleim ähnlich
Mundschleim: dick, sehr zäh, fadenziehend
Gemischter Speichel: ein wenig fadenziehend, von geringer Viskosität.

Wissenswertes zum Speichel

-- Produktion: bis 1.5 L/d, Fluss: 0.3-0.5 / 1.0-1.5 mL/min

-- Parotis(1) ~20%, Submandibularis(2) ~70%, Sublingualis(3) ~5%

-- Ruhesekretion: **pH 5.8 - 6.4**, Stimulation: bis 7.8

-- "Zutaten":

H_2O 99%
Enzyme: **Amylase**
Mucine
IgA u. a. Ig
Elektrolyte: Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}
bei niedrigem Fluss: hypoton
bei hohem Fluss: isoton

Vor- und Nachteile des Speichels

Vorteile:

•Probengewinnung

- Geringere Manipulationsmöglichkeit
- Privatssphäre wird gewahrt (größere Akzeptanz der Patienten)
- Nicht invasiv (kein medizinisches Fachpersonal notwedig)

•Relativ “saubere” Matrix (abhängig vom Entnahmesystem)

•Muttersubstanzen oftmals gut detektierbar

Nachteile:

•Probenmenge (Schwierigkeiten bei einigen Patientengruppen → Xerostomie)

•Einflussfaktoren auf die Analytkonzentration (Konzentrationsänderung durch Anregung des Speichel-flusses, unspezifische Bindung im Entnahmesystem)

Wie gelangen Drogen in den Speichel?

-- Orale Kontamination

-- aus dem Blut durch **passive Diffusion**

-- aktive Sekretion

-- Filtration

Faktoren mit Einfluß auf das S/P-Ratio:
-- **pKa** einer Substanz (sauer - alkalisch?)
-- Lipidlöslichkeit
-- **Plasmaprotein Bindung**
-- Molekulargewicht

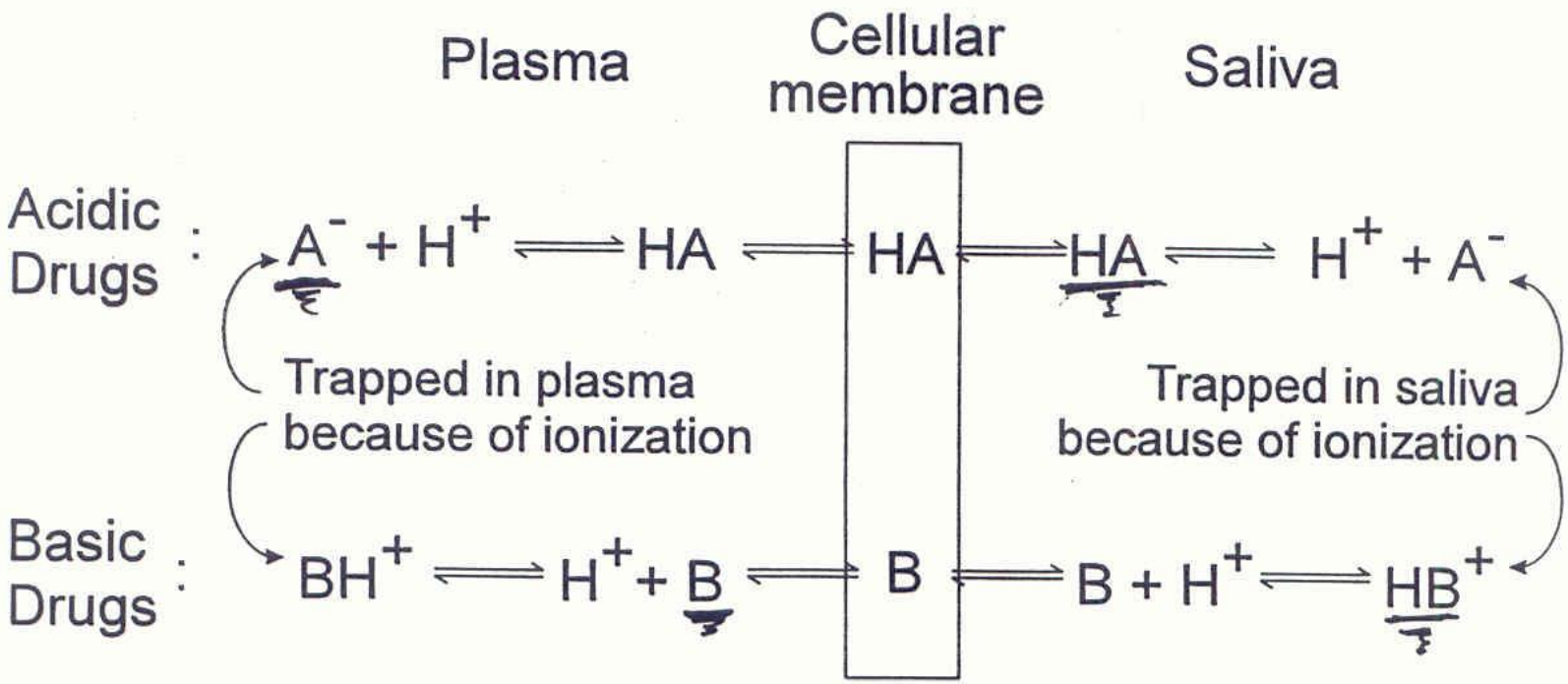
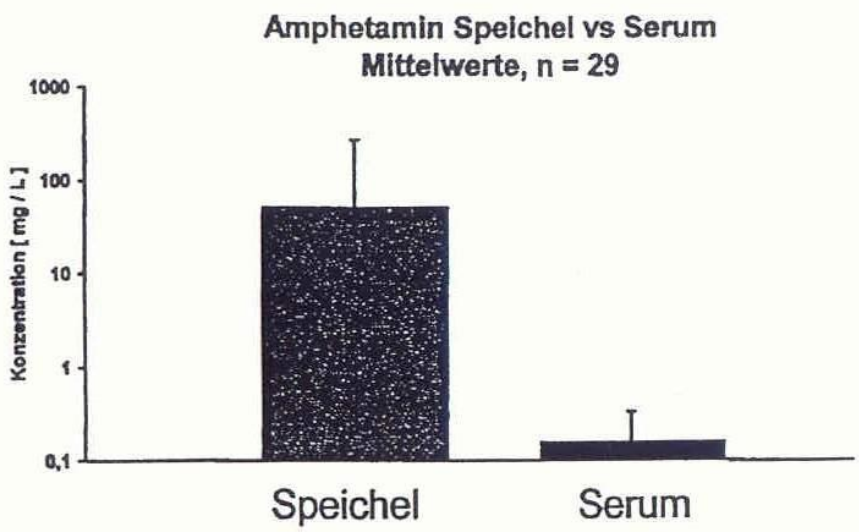
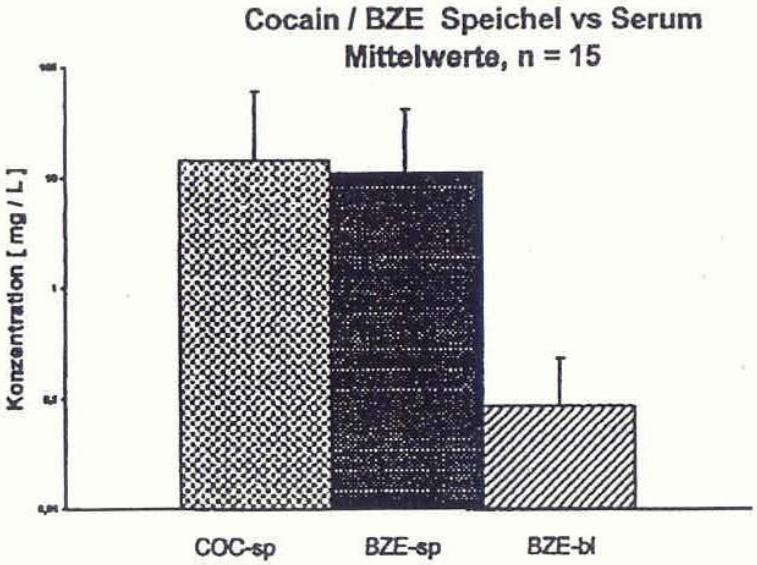
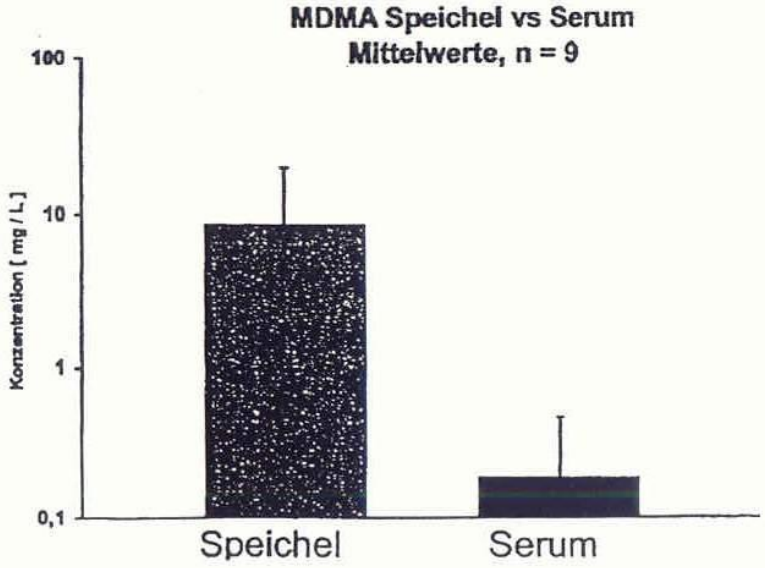
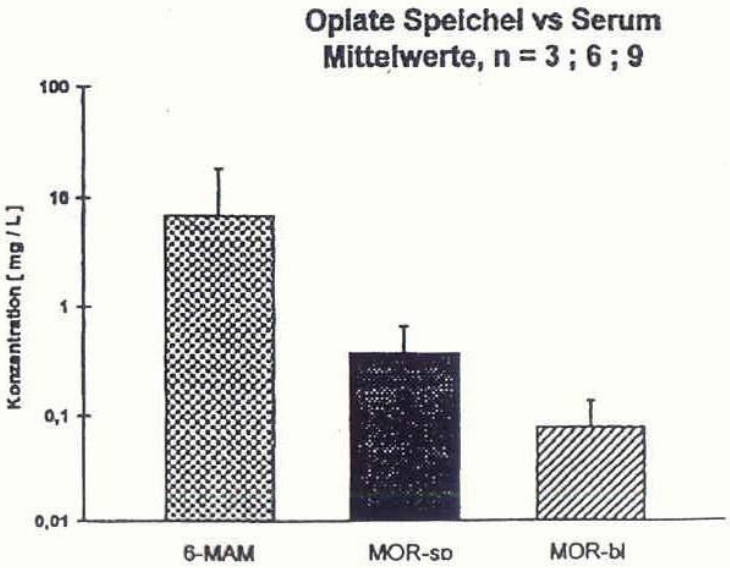


Fig. 1. Schematic diagram for transport of drugs into saliva or sweat.









Kauert, Blutalkohol 37, 2000

Journal of Analytical Toxicology, Vol. 35, January/February 2011

Oral Fluid is a Viable Alternative for Monitoring Drug Abuse: Detection of Drugs in Oral Fluid by Liquid Chromatography–Tandem Mass Spectrometry and Comparison to the Results from Urine Samples from Patients Treated with Methadone or Buprenorphine

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Abstract

Oral fluid is an alternative biological matrix that might have advantages over urine for drug analysis in treatment programs. A liquid chromatography–tandem mass spectrometry (LC–MS–MS) method has been used for screening 32 of the most commonly abused drugs and their metabolites in 0.5 mL preserved oral fluid, and the results were compared to results obtained from urine samples taken at the same time. In all, 164 pairs of oral fluid and urine were obtained from 45 patients stabilized on either methadone or buprenorphine. The total number of detections of drugs other than buprenorphine or methadone was 535 in oral fluid and 629 in urine. Morphine was found more often in urine (n = 66) than in oral fluid (n = 48), whereas the opposite was the case for 6-monoacetylmorphine (n = 20 in urine and n = 48 in oral fluid). Methadone showed the same detection frequency in urine and oral fluid (n = 75), whereas amphetamine (n = 45 in urine and n = 51 in oral fluid), methamphetamine (n = 39 in urine and n = 45 in oral fluid), and N-desmethyldiazepam (n = 37 in urine and n = 51 in oral fluid) were detected slightly more often in oral fluid. The other benzodiazepines, cannabis and cocaine were found more frequently in urine samples. If using a sensitive LC–MS–MS technique, oral fluid might be a good alternative to urine for detection of relatively recent use of drugs.

imens has accelerated over the last decade (1). An advantage with urine samples might be that drug ingestion can be detected for several days, and even weeks later, mainly because of detection of drug metabolites (2–4). However, urine may be difficult to collect; supervision intrudes on donors' privacy; the detection of drugs might be affected by, for example, the dilution of the urine due to fluid intake prior to urine sampling; and adulteration of the urine might render the analytical results worthless. Thus, there has been a growing interest in the use of oral fluid as an alternative to urine, and major technological advances have been made, particularly over the last 10 years (1). Collection of oral fluid is inoffensive, rapid, noninvasive, and easy, and the risk of adulteration is considered to be lower (5). Because of improved analytical techniques with increased sensitivity, a large number of drugs can be analyzed simultaneously in small sample volumes (6).

Oral fluid is a mixture of saliva, gingival crevicular fluid, cellular debris, and other components (5). Healthy adult subjects normally produce 500–1500 mL of oral fluid per day, at a rate of approximately 0.5 mL/min, but several physiological and pathological conditions can modify oral fluid production quantitatively and qualitatively (e.g., smell, taste stimulation, chewing, psychological and hormonal status, drugs, age, hered-

Table I. Cutoff Concentrations for Screening and Confirmation Analysis in Oral Fluid and Urine

Drug	Oral Fluid Analysis (ng/mL)	Urine Confirmation (ng/mL)	Urine Screening (ng/mL)
3-OH-Diazepam	3	150	
6-MAM	2	33	20
7-Aminoflunitrazepam	0.3	28	
7-Aminoclonazepam	1	29	
7-Aminonitrazepam	1	25	
Alprazolam	1	31	
α-OH-Alprazolam	NA*	32	
Amphetamine	1	135	300
Barbiturates			30
Benzodiazepines			200
Benzoylecgonine	14	58	
Bromazepam	16	32	
Buprenorphine	2		5
Buprenorphine-glucuronide	NA	5	
Norbuprenorphine-glucuronide	NA	12	
Cannabis			20
Carisoprodol†	52	1302	
Clonazepam	1	NA	
Codeine	3	60	
Cocaine	8	61	300
Diazepam	1	NA	
Fenazepam	2	3	
Flunitrazepam	1	NA	
Lorazepam	3	32	
LSD	0.3	0.03	0.50
MDA	36	1434	
MDEA	41	207	
MDMA	39	77	
Meprobamate†	44	1092	
Methadone	15	62	300
EDDP	NA	111	
Methamphetamine	3	149	
Morphine	6	29	
N-Desmethyldiazepam	1	135	
Nitrazepam	1	NA	
Opiates			300
Oxazepam	1	143	
THC-acid	0.3	10	
Zolpidem†	0.3	6	
Zopiclone†	2	4	

* Not analyzed.
† Only analyzed in urine if detected in oral fluid.

-- Intercept
-- LLE

Table II. Comparison of the Results from Oral Fluid and Urine Showing that the Results from the Sample Pairs Primarily Correspond

Drug	Positive OF* and Urine	Negative OF and Urine	Corresponding Results OF and Urine	Positive OF Only	Positive Urine Only
3-OH-Diazepam	6	117	123 (75%)	0	41
6-MAM	19	115	134 (82%)	29 !	1
7-Aminonitrazepam	9	149	158 (96%)	0	6
7-Aminoflunitrazepam	59	83	142 (87%)	3	19
7-Aminoclonazepam	26	122	148 (90%)	2	14
Alprazolam	9	153	162 (99%)	0	2
Amphetamine	45	113	158 (96%)	6 !	0
Benzoylecgonine	1	158	159 (97%)	0	5
Buprenorphine	67	–†		–	22 ?
Codeine	34	122	156 (95%)	4	4
Cocaine	0	161	161 (98%)	2	1
Methadone	75	89	164 (99%)	–	0
Methamphetamine	39	119	158 (96%)	6 !	0
Morphine	45	95	140 (85%)	3	21
N-Desmethyldiazepam	35	111	146 (89%)	16 !	2
Oxazepam	41	71	112 (68%)	9	43 !
THC/THCCOOH‡	81	64	145 (88%)	1	18
Zopiclone	4	106	110 (99%)	1	0

* Oral fluid.
† There were analytical problems with the oral fluid analysis.
‡ THC was analyzed in oral fluid, and THCCOOH was analyzed in urine.

DE GRUYTER OPEN

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Scandinavian journal of FORENSIC SCIENCE Nordisk rettsmedisin

Very long Detection Times after High and repeated intake of Heroin and Methadone, measured in Oral Fluid

Vindenes V[1*], Enger A[2], Nordal K[1], Johansen U[1], Christophersen AS[1], Øiestad EL[1]

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ABSTRACT

When detection times for psychoactive drugs in oral fluid are reported, they are most often based on therapeutic doses administered in clinical studies. Repeated ingestions of high doses, as seen after drug abuse, are however likely to cause positive samples for extended time periods. Findings of drugs of abuse in oral fluid might lead to negative sanctions, and the knowledge of detection times of these drugs are important to ensure correct interpretation. The aim of this study was to investigate the detection times of opioids in oral fluid. 25 patients with a history of heavy drug abuse admitted to a detoxification ward were included. Oral fluid and urine were collected daily and, if the patient gave consent, a blood sample was drawn during the first five days after admission. Morphine, codeine and/or 6-monoacetyl morphine (6-MAM) were found in oral fluid and/or urine from 20 patients. The maximum detection times in oral fluid for codeine, morphine and 6-MAM were 1, 3 and 8 days, respectively. Positive oral fluid samples were interspersed with negative samples, mainly for concentrations around cut off. Elimination curves for methadone in oral fluid were found for two subjects, and the detection times were 5 and 8 days. Oral fluid is likely to become a good method for detection of drug abuse in the future.

Keywords:

Oral fluid, opioid, detection time, heroin, methadone

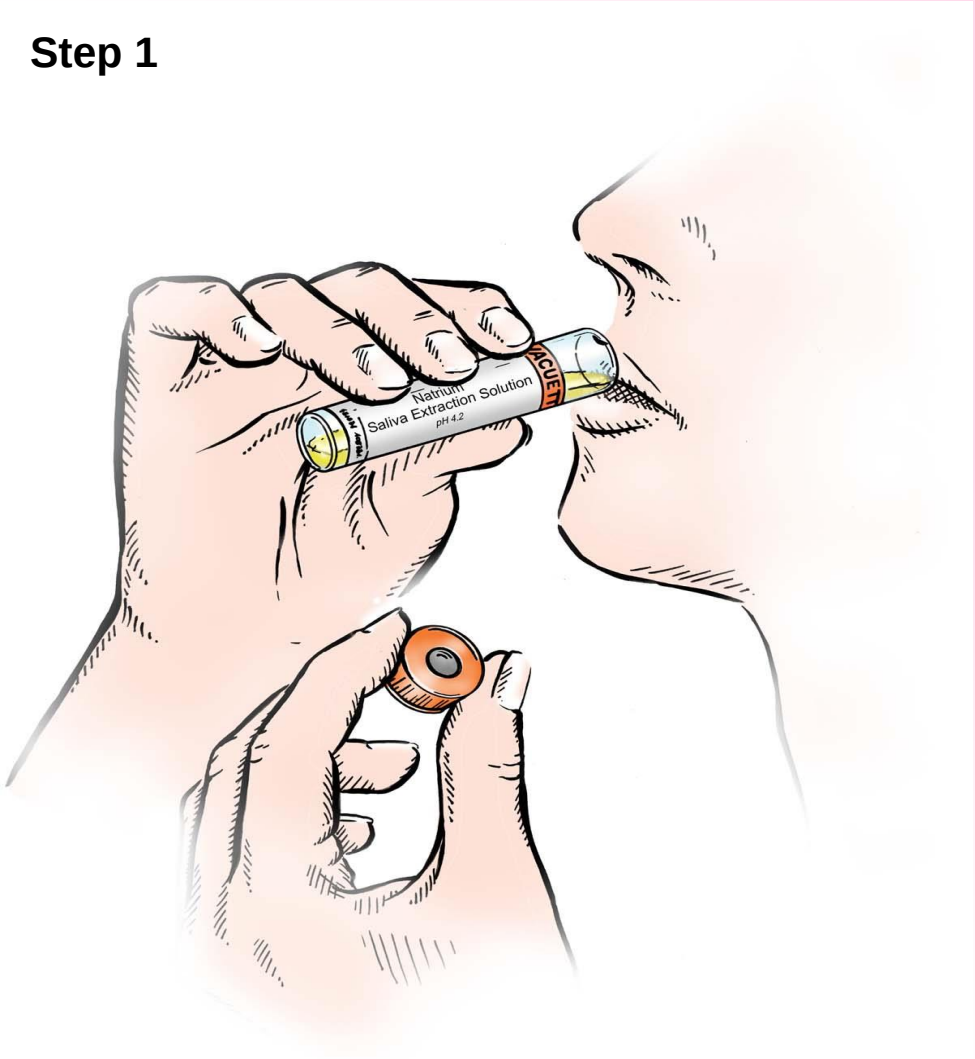
"**Saliva** is not one of the popular body fluids. It lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears."

ID Mandel
J. Oral Pathological Medicine, 1990

Saliva Collection System (SCS) pH 4.2
Greiner Bio-One

OF sampling with the Greiner Saliva Collection System:

Step 1

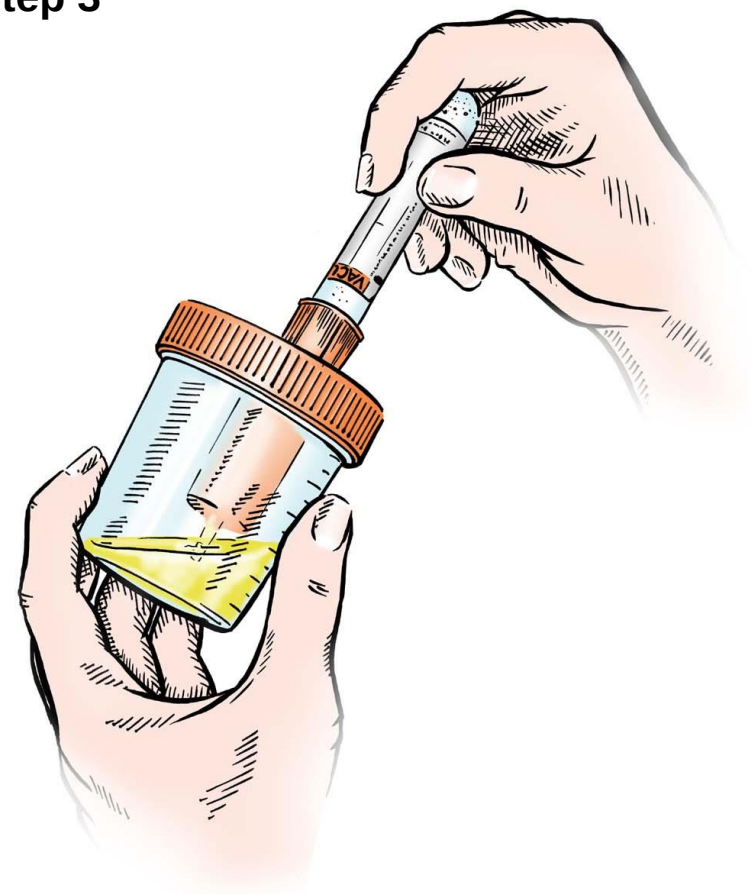


Rinsing of the oral cavity with Saliva Extraction Solution for 2 minutes

Step 2:

Spitting of the extracted oral fluid into the Saliva Collection Beaker

Step 3



Transfering of the extracted OF into the evacuated Saliva Collection Tubes

%OF is determined by photometry on Olympus AU680

always A + B sample!

Advantages:

-- quick (Xerostomia!), standardized time

-- acidic pH during collection keeps pH difference to plasma

-- acidic pH: 6-AM, Cocaine, Zopiclone etc. are stable

-- aqueous matrix: less ion suppression, rapid SALLE possible

Vorteile des Greiner Bio-One Speichelsammelsystems

- Anregung des Speichelflusses (Xerostomie als UAW vieler Psychopharmaka und Opiate/Opioide)
- ausreichendes Probenvolumen
- standardisierte Sammelzeit
- konstant saurer pH-Wert während des Sammelvorgangs (“Ionenfalle” für basische Substanzen)
- konstant saurer pH-Wert während der Lagerung (bessere Stabilität vieler Substanzen, z.B. “Badesalze”, Zopiclon oder 6-AM)
- A+B Probe entstammen demselben Sammelvorgang

Perianalytik

Perianalytik bezeichnet Überprüfung der Probe vor der eigentlichen Analytik (Echtheit, Verdünnung, Manipulationen etc.)

1. Probenvolumen

2. Photometrische Bestimmung des Speichelanteils (optische Dichte des Tartrazins)

3. Photometrische Bestimmung der Alpha-Amylase Konzentration durch die Messung der Alpha-Amylase Aktitvität (1. Authentizitätsmarker)

4. Bestimmung der Cortisolkonzentration (2. Authentiziätsmarker) mit UPLC-MS/MS

– Böttcher M., Preidel A., Beck O. “Peri-analytics” reference ranges for drug screening in oral fluid using the Greiner Bio-One collection device”; Poster: TIAFT, Bonn (2010)

Routine Drogenscreening im Speichel mit UPLC-MS/MS: Analyten

Analytes in „**Module A“,** cutoff 0.1-1 ng/mL neat OF, IS = 0.5 ng/mL SA/SES:

- **Peri-analytics:** volume, % saliva in SES, Amylase (Roche AU680), Cortisol (incl. in LC-MS/MS)

- **Substitution drugs:** D-/L-Methadone, EDDP, Buprenorphine, Norbuprenorphine

- **Amphetamines:** Amphetamine, Methamphetamine, MDMA, MDA, MBDB, BDB, MDEA, PMMA, Butylone, Mephedrone, Methylone, MDPV, alpha-PVP, 4-Methylethcatinon, Pentedron

- **Benzodiazepines**: Diazepam, Nordiazepam, Oxazepam, Midazolam, Flurazepam, Desalkyl-flurazepam, Temazepam, 7-Aminoclonazepam, Alprazolam, Flunitrazepam, 7-Aminofluni-trazepam, Bromazepam, Lorazepam, Phenazepam

- **Cocaine:** Cocaine, Benzoylecgonine, Methylecgonine, Lidocaine

- **Opiates:** Morphine, Codeine, **6-Acetylmorphine**, 6-Acetylcodeine, Norcodeine, Dihydrocodeine

- **Opioids:** Naloxone, Tilidine, Tramadol, O-Desmethyltramadol, Oxycodone, Noroxycodone, Fentanyl, Nortilidine, Hydromorphone, Noscapine, Loperamid, Dextromethorphan

- **Cannabinoids:** THC

- **Others:** Zolpidem, Zopiclone**,** Zaleplone, Ketamine, Methylphenidate, Ritalinic acid, Pregabalin, Gabapentin, Bupropion, Diphenhydramin

actual: N = 66 (3 transitions) **+ 62 deuterated IS** (2 transitions)

1st Study: is OF of equal value?

Drug abuse testing of patients in substitution therapy: UPLC-MS/MS screening in OF vs. urine testing with EIA

-- three month observation period

-- urine cutoffs: Amphs 500 ng/mL, Benzos (enzym. hydrolysis) 100 ng/mL, Coca 50 ng/mL, Opi 100 ng/mL, EDDP 100 ng/mL, Bupre 2 ng/mL, THC-COOH 25 ng/mL.

-- saliva cutoffs: 0.1-1 ng/mL (neat OF)

-- Patients from:

1. an outpatient clinic **(OPC)** where the drug testing was stepwise moved from urine to SA.
   - **194 patients** (26 Bupre, 67 Metha, 101 Pola), **902 SA** samples.
   - **182 patients** (25 Bupre, 66 Metha, 91 Pola), **1119 urine** samples.

2. other outpatient clinics **(ALL)** with more random selection between the two matrices.
   - **612 patients** from 23 clinics (116 Bupre, 265 Metha, 231 Pola), **1072 SA** samples.
   - **1463 patients** from 40 clinics (285 Bupre, 673 Metha, 505 Pola), **9008 urine** samples.

Drug abuse testing of patients in substitution therapy: UPLC-MS/MS screening in saliva vs. urine testing with EIA

	OPC	OPC	OPC	ALL	ALL	ALL
	saliva % pos. spls.	urine % pos. spls.	urine no. of spls.	saliva % pos. spls.	urine % pos. spls.	urine no. of spls.
Amphetamines	9.3	3.3	1082	10.3	4.1	7396
Benzodiazepines	11.0	14.4	958	25.7	22.4	6891
Cocaine	5.2	3.9	1075	9.8	7.2	8295
Opiates	13.5	13.5	968	17.6	21.7	6977
Methadone saliva EDDP urine	86.6	85.2	953	85.4	88.0	8938
THC	26.9	-	-	30.5	31.3	598
Opioids	1.2	-	-	2.1	-	-
Others	0.8	-	-	1.4	-	-
Buprenorphine	12.3	-	-	16.9	73.1	640
	n = 902			**n = 1072**		

Methadone/EDDP was positive in both matrices where expected.
However, Buprenorphine was negative in 8 OF samples from 2 OPC patients in low dose therapy (0.4 and 1.0 mg/d).
Cutoff 0.1 ng/mL?

Detailed results OF

	OPC	OPC	ALL	ALL
	saliva % pos. spls.	saliva % from pos.	saliva % pos. spls.	saliva % from pos.
Opiates	13.5	100	17.6	100
Morphine	13.3	98.4	16.9	95.8
6-Acetylmorphine	10.3	**76.2**	13.4	**76.2**
Codeine	8.2	60.7	12.8	72.5
6-Acetylcodeine	3.7	**27.0**	4.8	27.0
Norcodeine	2.8	20.5	4.1	23.3
Dihydrocodeine	0.1	0.8	0.1	0.5
Methadone saliva **EDDP** urine	86.6	100	85.4	100
THC	26.9	100	30.5	100
Opioids	1.2	100	2.1	100
Fentanyl	-	-	0.5	21.7
Tramadol	0.8	63.6	0.8	34.8
Tilidine	0.2	18.2	0.4	17.4
Naloxone	0.2	18.2	0.4	17.4
Oxycodone	-	-	0.2	8.7
Others	0.8	100	1.4	100
Ketamine	0.7	85.7	0.6	40.0
Zopiclone	0.1	14.3	0.4	26.7
Zolpidem	-	-	0.3	20.0
Methylphenidate	-	-	0.2	13.3
Buprenorphine	12.3	100	16.9	100

2nd study: Cutoff considerations
All routine OF sampels, 3 month

Samples: 5355

from pats. in maintenance therapy:	4954 spls. = 92.5% of all spls.
from Methadone/Polamidone™ pats.:	3671 spls. = 68.5% of all spls.
from Buprenorphine pats.:	1283 spls. = 24.0% of all spls.

Patients: 2050

male: 1455 (71.0%), female: 595 (29.0%)

in maintenance therapy:	1877 pats. = 91.6% of all pats.
male:	1347 pats. = 65.7% of all pats.
female:	530 pats. = 25.9% of all pats.
Methadone/Polamidone™ pats.:	1315 pats. = 64.1% of all pats.

male: 924 (63.5%), female: 391 (36.5%)

Buprenorphine pats.:	562 pats. = 27.5% of all pats.

male: 423 (75.3%), female: 139 (24.7%)

Opiates :

CO 1 ng/mL: 610 pos. samples = 11.4% **CO 10 ng/mL: 397 pos. samples = 7.4%**

a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 34.9%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
597	Morphine	376	Morphine	37.0%
494	6-Acetylmorphine	237	6-Acetylmorphine	52.0%
396	Codeine	217	Codeine	45.2%
173	6-Acetylcodeine	100	6-Acetylcodeine	42.2%
129	Norcodeine	10	Norcodeine	92.2%
11	Dihydrocodeine	6	Dihydrocodeine	45.2%

81.0% of all Opiate positive samples contained 6-Acetylmorphine thus proving Heroin abuse.

34.7% of all 6-Acetylmorphine positive samples contained 6-Acetylcodeine thus proving "Street Heroin" abuse.

60.0% of all Opiate positive samples contained 6-Acetylmorphine thus proving Heroin abuse.

42.2% of all 6-Acetylmorphine positive samples contained 6-Acetylcodeine thus proving "Street Heroin" abuse.

3rd study: smoked heroin?

2814 oral fluid samples

1875 patients

406 OF samples
opiates positive = 14.4%

from 314 pats. = 16.7%

314 OF samples
6-AM positive = 77.4%
from 246 pats. = 78.3%
Heroin abuse proven

- Heroin could be detected in 35 opiates-positive samples (8.6%) from 34 pats. (10.8%). Heroin concentrations ranged from 0.2 to 155 ng/mL (mean = 10.7 ng/mL, median = 3.1 ng/mL). All samples with 6-AM concentrations >20 ng/mL.
- All samples with 6-AC contained also 6-AM.

35 OF samples
Heroin pos. = 11.2%
of 6-AM positive samples
= 8.6% of opiates-positive samples
Oral contamination
= smoked heroin?

156 OF samples
6-AC positive = 49.6%
of 6-AM positive samples
= 38.4% of opiates-positive samples
Street-heroin abuse proven

Multi-Target-Screening Speichel

MVZ Labor Dessau GmbH

Geschäftsführer:
Dr. med. Juliane Böttcher-Lorenz

Bauhüttenstraße 6, 06847 Dessau

Drogen- und Medikamentenanalytik
Dr. rer. medic. M. Böttcher

Telefon : 0340/5405372
Funk : 0162/9071483

==========
Befundbericht
- Archivbd. -
==========
Blatt 1

10827 Berlin

Name des Patienten:	Gesch.	Geburtsdatum:	Kasse:	Eingangsdatum / Tagesnummer:
			K	04.05.2015 /SP000232
ID-Nr.: 0606205623	Ext.-Nr.:			Befundausgang: 05.05.15

Material: Speichel

Sonstige Medikation: Substitol

Drogen-/Medikamentenscreening
im Speichel mit LC/MS-MS
Entnahmesystem: Greiner

Volumen A-Probe (Speichel/SES)	2.5	ml	
Volumen B-Probe (Speichel/SES)	1.0	ml	

Das Gesamtvolumen von A- und B-Probe sollte 2 - 7 ml betragen.

Speichelanteil in der Probe:	50.9	%	(20 - 80)
Amylase im Speichel:	127866	U/l	(> 10000)
Cortisol im Speichel:	2.4	ng/ml	(1.0 - 6.0)

Bei Cortisolsuppression durch entsprechende Substanzen wie z. B. Methadon werden Cortisolkonzentrationen von 0.1 - 6.0 ng/ml gemessen.

L-/D-Methadon:	negativ	ng/ml	(Cutoff 1)
Buprenorphin:	negativ	ng/ml	(Cutoff 0.1)
Amphetamine und Derivate:	negativ	ng/ml	(Cutoff 1)

Berücksichtigte Substanzen:

D-/L-Amphetamin	MDA	BDB
D-/L-Methamphetamin	MDEA	MDPV
MDMA	MBDB	
Butylon	Mephedron	Methylon

Benzodiazepine:	negativ	ng/ml	(Cutoff 0.1-1)

Berücksichtigte Substanzen:

Diazepam	Temazepam	Midazolam
Nordiazepam	Alprazolam	7-Aminoclonazepam
Oxazepam	Lorazepam	Bromazepam
Flurazepam	Flunitrazepam	7-Aminoflunitrazepam
Desalkylflurazepam		

Kokain:	negativ	ng/ml	(Cutoff 1)

Berücksichtigte Substanzen:

- Fortsetzung siehe nächstes Blatt -

DAkkS Deutsche Akkreditierungsstelle D-ML-13316-01-00 D-PL-13316-01-00

MVZ Labor Dessau GmbH

Geschäftsführer:
Dr. med. Juliane Böttcher-Lorenz

Bauhüttenstraße 6, 06847 Dessau

Drogen- und Medikamentenanalytik
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Telefon : 0340/5405372
Funk : 0162/9071483

==========
Befundbericht
- Archivbd. -
==========
Blatt 2

10827 Berlin

Name des Patienten:	Gesch.	Geburtsdatum:	Kasse:	Eingangsdatum / Tagesnummer:
			K	04.05.2015 /SP000232
ID-Nr.: 0606205623	Ext.-Nr.:			Befundausgang: 05.05.15

Kokain	Benzoylecgonin	Lidocain

Opiate:	POSITIV		ng/ml	(Cutoff 1)

Berücksichtigte Substanzen:

Morphin	Codein	6-Acetylcodein
6-Acetylmorphin	Norcodein	Dihydrocodein

Morphin:	>20	POS	ng/ml	(Cutoff: 1)
Opioide:	POSITIV		ng/ml	(Cutoff 0.1-1)

Berücksichtigte Substanzen:

Naloxon	Tilidin	Fentanyl
Tramadol	Nortilidin	Oxycodon
o-Desmethyltramadol	Hydromorphon	Noroxycodon

Hydromorphon:	1.3	POS	ng/ml	(Cutoff: 1)

Bitte telefonische Rücksprache
Dr. M. Böttcher
(Durchwahl: 0340 5405372 o. 0162 9071483)

Cannabis:	negativ	ng/ml	(Cutoff 1)

Berücksichtigte Substanzen:
THC

Sonstige:	negativ	ng/ml	(Cutoff 0.1-2)

Berücksichtigte Substanzen:

Zolpidem	Zaleplon	Methylphenidat
Zopiclon	Ketamin	Ritalinsäure
Pregabalin	Gabapentin	Bupropion

Befund medizinisch validiert Dr. Böttcher

Archivbefund vom 12.05.15 , Endbefund vom 05.05.15

Nicht budgetrelevant, da Ausnahmeindikation vorhanden.

DAkkS Deutsche Akkreditierungsstelle D-ML-13316-01-00 D-PL-13316-01-00

Multi-Target-Screening Speichel

Opiate:		**POSITIV**	ng/ml	(Cutoff 1)
Berücksichtigte Substanzen:				
Morphin	Codein	6-Acetylcodein		
6-Acetylmorphin	Norcodein	Dihydrocodein		

Morphin:	>20	POS	ng/ml	(Cutoff: 1)

Opioide:		**POSITIV**	ng/ml	(Cutoff 0.1-1)
Berücksichtigte Substanzen:				
Naloxon	Tilidin	Fentanyl		
Tramadol	Nortilidin	Oxycodon		
o-Desmethyltramadol	Hydromorphon	Noroxycodon		

Hydromorphon:	1.3	POS	ng/ml	(Cutoff: 1)
Bitte telefonische Rücksprache				
Dr. M. Böttcher				

Multi-Target-Screening Speichel

SUBSTITUTIONSPATIENT MIT METHADON

MVZ Labor Dessau GmbH

Geschäftsführer:
Dr. med. Juliane Böttcher-Lorenz

Bauhüttenstraße 6, 06847 Dessau

Drogen- und Medikamentenanalytik
Dr. rer. medic. M. Böttcher

Telefon : 0340/5405372
Funk : 0162/9071483

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Befundbericht
- Archivbd. -
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Blatt 1

Name des Patienten:	Gesch.	Geburtsdatum:	Kasse:	Eingangsdatum / Tagesnummer:
	M	26.03.63	K	13.10.2014 /SP000042

ID-Nr.: 0604808444 Ext.-Nr.: Befundausgang: 14.10.14

Material: Speichel

Tagesdosierung Methadon:	8		ml	
Drogen-/Medikamentenscreening im Speichel mit LC/MS-MS				
Entnahmesystem: Greiner				
Volumen A-Probe (Speichel/SES)	2.0		ml	
Volumen B-Probe (Speichel/SES)	0.4		ml	

Das Gesamtvolumen von A- und B-Probe sollte 2 - 7 ml betragen.

Speichelanteil in der Probe:	19.0		%	(20 - 80)
Amylase im Speichel:	389905		U/l	(> 10000)
Cortisol im Speichel:	4.2		ng/ml	(1.0 - 6.0)

Bei Cortisolsuppression durch entsprechende Substanzen wie z. B. Methadon werden Cortisolkonzentrationen von 0.1 - 6.0 ng/ml gemessen.

L-/D-Methadon:	POSITIV		ng/ml	(Cutoff 1)
Methadon (Messwert):	>20	POS	ng/ml	(Cutoff: 1)
EDDP:	6.6	POS	ng/ml	(Cutoff: 0.1)
Buprenorphin:	negativ		ng/ml	(Cutoff 0.1)

Amphetamine und Derivate: negativ ng/ml (Cutoff 1)

Berücksichtigte Substanzen:

D-/L-Amphetamin	MDA	BDB
D-/L-Methamphetamin	MDEA	MDPV
MDMA	MBDB	
Butylon	Mephedron	Methylon

Benzodiazepine: negativ ng/ml (Cutoff 0.1-1)

Berücksichtigte Substanzen:

Diazepam	Temazepam	Midazolam
Nordiazepam	Alprazolam	7-Aminoclonazepam
Oxazepam	Lorazepam	Bromazepam
Flurazepam	Flunitrazepam	7-Aminoflunitrazepam
Desalkylflurazepam		

- Fortsetzung siehe nächstes Blatt -

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Befundbericht
- Archivbd. -
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Blatt 2

Name des Patienten:	Gesch.	Geburtsdatum:	Kasse:	Eingangsdatum / Tagesnummer:
	M	26.03.63	K	13.10.2014 /SP000042

ID-Nr.: 0604808444 Ext.-Nr.: Befundausgang: 14.10.14

Kokain: negativ ng/ml (Cutoff 1)

Berücksichtigte Substanzen:

Kokain	Benzoylecgonin	Lidocain

Opiate: POSITIV ng/ml (Cutoff 1)

Berücksichtigte Substanzen:

Morphin	Codein	6-Acetylcodein
6-Acetylmorphin	Norcodein	Dihydrocodein

Morphin:	>20	POS	ng/ml	(Cutoff: 1)
6-Acetylmorphin:	>20	POS	ng/ml	(Cutoff: 1)
Codein:	>20	POS	ng/ml	(Cutoff: 1)
Norcodein:	5.1	POS	ng/ml	(Cutoff: 1)
6-Acetylcodein:	9.5	POS	ng/ml	(Cutoff: 1)

Opioide: negativ ng/ml (Cutoff 0.1-1)

Berücksichtigte Substanzen:

Naloxon	Tilidin	Fentanyl
Tramadol	Nortilidin	Oxycodon
o-Desmethyltramadol	Hydromorphon	Noroxycodon

Cannabis: negativ ng/ml (Cutoff 1)

Berücksichtigte Substanzen:
THC

Sonstige: negativ ng/ml (Cutoff 0.1-2)

Berücksichtigte Substanzen:

Zolpidem	Zaleplon	Methylphenidat
Zopiclon	Ketamin	Ritalinsäure
Pregabalin	Gabapentin	Bupropion

Befund medizinisch validiert Dr. Böttcher

Archivbefund vom 15.10.14 , Endbefund vom 14.10.14

Nicht budgetrelevant, da Ausnahmeindikation vorhanden.

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Multi-Target-Screening Speichel

SUBSTITUTIONSPATIENT MIT METHADON

L-/D-Methadon:	POSITIV		ng/ml	(Cutoff 1)
Methadon (Messwert):	>20	POS	ng/ml	(Cutoff: 1)
EDDP:	6.6	POS	ng/ml	(Cutoff: 0.1)

- Substitution mit Methadon
  - Methadon positiv
  - EDDP positiv

Opiate:	POSITIV		ng/ml	(Cutoff 1)

Berücksichtigte Substanzen:

Morphin	Codein	6-Acetylcodein
6-Acetylmorphin	Norcodein	Dihydrocodein

Morphin:	>20	POS	ng/ml	(Cutoff: 1)
6-Acetylmorphin:	>20	POS	ng/ml	(Cutoff: 1)
Codein:	>20	POS	ng/ml	(Cutoff: 1)
Norcodein:	5.1	POS	ng/ml	(Cutoff: 1)
6-Acetylcodein:	9.5	POS	ng/ml	(Cutoff: 1)

- Beweis von 6-Acetylcodein
  - Missbrauch von Straßenheroin

6-AM in breath?

Vielen Dank für Ihre Aufmerksamkeit !

michael.boettcher@laborpraxis-dessau.de