



VICTORIAN INSTITUTE OF FORENSIC MEDICINE

Alere™ DDS®2 Mobile Analyser Assessment

**DDS®2-403 (6-panel) Test Kit
Performance Report**

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Background

A standard protocol developed in the toxicology section of the Victorian Institute of Forensic Medicine has been used to test the Alere™ DDS®2 Mobile Analyser with the DDS®2-403 (6-panel) test kit for performance around the manufacturer's nominated detection limits to presumptively test oral fluid (saliva) for the presence of drugs of abuse.

The Alere™ DDS®2 Mobile Analyser was supplied with DDS®2-403 (6-panel) test kits designed to detect the presence of amphetamine, benzoylecgonine, methamphetamine, morphine, temazepam and THC in oral fluid. The nominated reader cut-offs of the DDS®2-403 test kit are shown in Table 1.

Table 1. Nominated cut-offs by the manufacturer

<i>Test Drug</i>	<i>Test Strip</i>	<i>DDS®2-403 Cut-off</i>
d-Methamphetamine	methamphetamines	50ng/mL
(-)-Δ ⁹ -THC	cannabis	25ng/mL
d-Amphetamine	amphetamines	50ng/mL
Benzoylecgonine	cocaine	30ng/mL
Morphine	opiates	40ng/mL
Temazepam	benzodiazepines	20ng/mL

Strategy and Testing Regimens

The testing comprised of the following parts:

Part A. DDS®2-403 test kits were evaluated for specificity (false-positives) to each of the 6 chemistries.

Part B. DDS®2-403 test kits were evaluated for sensitivity and compliance to nominated cut-offs for each of the 6 target analytes.

Materials

Two DDS®2 Mobile Analysers were used during the study.

Serial numbers were 10003365 (Device 1 - #1440) and 10003358 (Device 2 - #1339).

The test kit batches used in the evaluation were:

- Ref# DDS®2-403, Lot# 101271, expiry 2016/09.

Test Cassettes used in evaluation were:

- Ref# DDS®2-403, Lot# 101373, expiry 2016/09.

Buffer cartridges in this batch were integrated with the therefore retain the same details as listed above.

Oral Fluid Collection Devices used in evaluation were:

- Ref# ANA126, Lot# 101271/1, expiry 2017/05.

Collection devices were packaged together in the kit with test cassettes.

Fresh certified liquid reference standards were purchased from Lipomed with catalogue number, lot number and expiry dates listed in Table 2. A liquid reference standard of temazepam was unable to be sourced due to the time constraints for evaluation. The laboratory's certified stock was used and a standard solution was prepared according to VIFM's standard procedure on the day of testing.

Table 2. Drug standards used in the study

<i>Drug name</i>	<i>Concentration</i>	<i>Reference code</i>	<i>Lot number</i>	<i>Expiry</i>
d-Methamphetamine.HCl	1mg free base/mL	AMP-732-HC-1LM	732.1B3.1L1	2016/09
(-)-Δ ⁹ THC	1mg/mL	THC-135-1LE	135.1B69.0L4	2017/03
d-Amphetamine.HCl	1mg free base/mL	AMP-96-HC-1LM	96.1B10.1L1	2017/05
Benzoylecgonine	1mg /mL	COC-204-FB-1LM	204.1B13.3L1	2015/12
Morphine.monohydrate	1mg free base/mL	M-35-FB-1LM	35.1B0.1L9	2017/04
Temazepam	1mg/mL	2500048 (Fabbrica Italiana Sintetici)	200305200125	n/a

Oral fluid (OF) was individually collected from 11 human drug-free volunteers and stored at -20°C overnight in accordance with the protocol specified by the manufacturer. After defrosting, the OF was pooled, mixed and centrifuged at 2000g for 20 minutes. The supernatant fraction of OF was then collected and used for all preparations.

The pooled OF was also analysed by a validated LC-MS/MS method to confirm it was negative for target and related compounds.

Methods

Part A. Evaluation of the DDS®2-403 test kits for specificity (false-positives).

Pooled blank OF (20 aliquots) were tested for each of the 6 chemistries using the same analysis conditions as defined by the manufacturer's operation manual. Responses from each of these kits/cartridges were recorded through the use of two DDS®2 Mobile Analysers.

Analysis protocols were as follows:

0.6mL each of OF was pipetted on the OF collector mouthpiece of the Oral Fluid Collection Device. Once the indicator had begun to change colour the collector was inserted into the port of the test cassette which had been placed in the DDS®2 Mobile Analyser, as per manufacturer's instructions. A positive (+) or negative (-) result was recorded as determined by the analyser. Printouts of each of the results were also retained.

Part B. Evaluation of the DDS®2-403 for sensitivity and compliance to nominated cut-offs.

Aliquots of pooled OF were then spiked at nominated concentrations of 50 % below ("LOW") and 50 % above ("HIGH") the stated DDS®2-403 cut-offs and analysed through the use of two DDS®2 Mobile Analysers.

The drug concentrations investigated are detailed in Table 3.

Table 3. Nominated spiked OF concentrations for DDS®2-403 test kits

<i>Test Drug</i>	<i>LOW (-50% of cut-off value)</i>	<i>HIGH (+50% of cut-off value)</i>
d-Methamphetamine	25ng/mL	75ng/mL
(-)-Δ ⁹ THC	12.5ng/mL	37.5ng/mL
d-Amphetamine	25ng/mL	75ng/mL
Benzoylcegonine	15ng/mL	45ng/mL
Morphine	20ng/mL	60ng/mL
Temazepam	10ng/mL	30ng/mL

Standards were prepared using VIFM's protocol and equipment (Appendix 1). Multi-spikes were prepared which were added to blank OF to achieve the above concentrations with a total volume of 1% methanol in the final spiked OF. Each concentration was tested 10 times on each of the two devices.

Analysis protocols were as follows:

0.6mL each of OF was pipetted on the OF collector mouthpiece of the Oral Fluid Collection Device. Once the indicator had begun to change colour the collector was inserted into the port of the test cassette which had been placed in the DDS®2 Mobile Analyser, as per manufacturer's instructions. A positive (+) or negative (-) result was recorded as determined by the analyser. Printouts of each of the results were also retained.

All fortified drug solutions in OF were confirmed by a validated LC-MS/MS method to ensure concentrations in the OF used were accurate.

The percentage positive response at each of the tested concentrations are summarised in the Results section.

Results

Part A. Evaluation of the DDS®2-403 for specificity (false-positives)

Data are shown below for each of the 6 chemistries using the test drug nominated at the concentrations listed in pooled OF. The final column shows the overall response rate for the replicate measurements per level as a percentage.

A) DDS®2-403 Blank pooled OF test (false-positive)

Drug	Test Concentration	Device 1 (serial no 10003365)										Device 2 (serial no 10003358)										% positive
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
d-Methamphetamine	nil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
(-)-Δ9 THC	nil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
d-Amphetamine	nil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Benzoylcegonine	nil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Morphine	nil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Temazepam	nil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%

The pooled blank OF did not give any positive responses to the 6 drugs during initial screening using the DDS®2-403 test kits.

Part B. Evaluation of the DDS®2-403 for sensitivity and compliance to nominated cut-offs**Bi) DDS®2-403 LOW concentration test (-50% of cut-off)**

Drug	Test Concentration	Device 1 (serial no 10003365)										Device 2 (serial no 10003358)										% positive
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
d-Methamphetamine	25ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	5%
(-)-Δ9 THC	12.5ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
d-Amphetamine	25ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Benzoylecgonine	15ng/mL	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	5%
Morphine	20ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Temazepam	10ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%

These data show that for OF spiked at 50% below the manufacturer's cut-off, no tests produced a positive result for THC, amphetamine, morphine and temazepam. Benzoylecgonine produced one positive result on device 1, while methamphetamine produced one positive result on device 2. Over 20 tests at 50% below the stated cut-off on the DDS®2-403 test kit resulted in an overall positive rate of 5% for both methamphetamine and benzoylecgonine.

Bii) DDS®2-403 HIGH concentration test (+50% of cut-off)

Drug	Test Concentration	Device 1 (serial no 10003365)										Device 2 (serial no 10003358)										% positive
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	

d-Methamphetamine	75ng/mL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
(-)-Δ9 THC	37.5ng/mL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
d-Amphetamine	75ng/mL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
Benzoylcegonine	45ng/mL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
Morphine	60ng/mL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%
Temazepam	30ng/mL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100%

At 50% higher than the manufacturer's cut-off for all drugs, 100% of tests returned a positive result on each device.

Conclusions

The device was found to be reliable with no failed tests of the 60 tests conducted throughout the study.

The results indicated that the DDS®2 Mobile Analysers and DDS®2-403 test kits performed well with regards to specificity (false-positives). No positive result was recorded for any of the six drug chemistries on the 20 tests performed with the pooled OF, and laboratory testing with a validated LC-MS/MS detection method confirmed no target or related compounds were present.

For the DDS®2-403 test kits, at 50% below the manufacturer's cut-off concentration, no positive tests were recorded for THC, amphetamine, morphine and temazepam while at 50% below manufacturer's cut off a 5% positive rate was determined for methamphetamine and benzoylecgonine.

All chemistries yielded a 100% detection rate for the DDS®2-403 test kits at 50% above the cut-off in accordance with the manufacturer's stated cut-off concentrations.

Appendix 1. VIFM protocol for preparation of spiked OF

150uL of each spike solution was added to 15mL of pooled OF giving 1% methanol in each concentration level. **DDS®2-403**
spike solutions

<i>Drug</i>	<i>OF target concentration</i>	<i>Concentration of stock used</i>	<i>Volume added</i>	<i>Volume of methanol added</i>	<i>Final spike concentration</i>
LOW test concentration (-50% of cut-off)					
d-Methamphetamine	45ng/mL	0.1mg/mL	45µL	892.5µL	4.5µg/mL
(-)-Δ9 THC	130ng/mL	0.1mg/mL	130µL		13.0µg/mL
d-Amphetamine	40ng/mL	0.1mg/mL	40µL		4.0µg/mL
Benzoylecgonine	17ng/mL	0.1mg/mL	17µL		1.7µg/mL
Morphine	15ng/mL	0.1mg/mL	15µL		1.5µg/mL
Temazepam	17ng/mL	0.1mg/mL	17µL		1.7µg/mL
HIGH test concentration (+50% of cut-off)					
d-Methamphetamine	135ng/mL	0.1mg/mL	135µL	677.5µL	13.5µL/mL
(-)-Δ9 THC	400ng/mL	0.1mg/mL	400µL		40,0µL/mL
d-Amphetamine	120ng/mL	0.1mg/mL	120µL		712.0µL/mL
Benzoylecgonine	52ng/mL	0.1mg/mL	52µL		5.2µL/mL
Morphine	45ng/mL	0.1mg/mL	45µL		4.5µL/mL
Temazepam	52ng/mL	0.1mg/mL	52µL		5.2µL/mL

