ichroma™ AMP/EDDP/OPI

INTENDED USE

ichroma™ AMP/EDDP/OPI is a fluorescence immunoassay (FIA) for the qualitative determination of amphetamine (AMP), 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP, methadone metabolite) and morphine (OPI) in human urine. It is useful as an aid in screening of three DOA items (AMP, EDDP, OPI).

For *in vitro* diagnostic use only.

INTRODUCTION

DOA (Drug of Abuse) contains drugs, psychotropic drugs, and marijuana, and are strictly controlled and managed by local regulations as misuse can cause addiction and irreversible serious mental and physical damage.[1)]

In the diagnosis of narcotics, on-site diagnosis is becoming important. *In vitro* immunodiagnostic technology using antigen antibody reactions is becoming a major method for field diagnosis application. Fluorescence-based analysis is a technology capable of precisely analyzing a fine amount of material in a sample. Among narcotics, AMP, EDDP, and OPI are the main substances that are misused and cause many social problems.[2), 3)]

Amphetamine (AMP): Amphetamine is known as a strong stimulant for the central nervous system. It is a sympathetic nerve stimulant and acts to increase the concentration of bioamines such as dopamine, norepinephrine, and serotonin in the synapse. It is mainly prescribed as a treatment for ADHD in children and adolescents, and is used as an improvement drug for daytime hyper sleep disorders associated with narcolepsy in adults. Overdose can cause psychosis, and can lead to nerve damage, halucination, paranoid delirium, seizures, coma and death.[4), 5)]

Methadone (EDDP): EDDP is a major metabolite in which methadone is metabolized in the body and released into urine. Methadone is prescribed as a painkiller, and although it has a different chemical structure from morphine, it acts on opioid receptors to produce almost the same effect and is prescribed for the purpose of alleviating and treating opioid addicts. It is a representative drug that causes death due to misuse. Restlessness, nausea or vomiting, slow breathing, itchy skin, heavy sweating, construction, and sexual problems are known as side effects of methadone.[6), 7)]

Morphine (OPI): Morphine is a representative opioid substance derived from poppy plants, and has been used as a drug for painkillers, sedatives, and antidiarrheals since ancient times. It acts as a powerful painkiller and is prescribed as an oral drug or injection under strict control for various diseases accompanying severe pain. There are addictive and withdrawal symptoms, and misuse can lead to side effects of sedation, dizziness, nausea, vomiting, construction, dependency, tolerance, and resistance depression.[8), 9)]

PRINCIPLE

The test uses a competitive immunoassay method.

The analytes in the sample bind to the fluorescence-labeled detector antibodies in buffer, forming the complexes as a sample mixture. They will migrate onto nitrocellulose matrix, which will interfere with the binding of the free fluorescence-labeled detector antibodies to the immobilized-carrier proteins-conjugated AMP, EDDP, and OPI on the test strip.

More analytes in the sample will result in less free detection antibodies to accumulate, which lead to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for ichroma™ tests to screen AMP, EDDP, and OPI in the sample.

COMPONENTS

ichroma™ AMP/EDDP/OPI consists of 'cartridges', detector tubes', 'detector diluents'.

- The cartridge contains the membrane called a test strip which includes three carrier proteins which are conjugated with AMP, EDDP, and OPI individually, and two control proteins. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has a granule containing fluorescence conjugates for detection of AMP, EDDP, OPI and sodium azide as a preservative in phosphate buffered saline. All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide as a preservative, and Tween 20 as a detergent in disodium phosphate, monosodium phosphate, and phosphate buffered saline (PBS), and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations.
- If test components and/or sample are stored in refrigerator, then allow the cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.

- Used cartridges, detector tubes, detector diluents and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- **ichroma™ AMP/EDDP/OPI** will provide accurate and reliable results subject to the below condition.
  - **ichroma™ AMP/EDDP/OPI** should be used only in conjunction with the instrument for ichroma™ tests.
  - The optimal operating temperature is at 35°C. Testing outside this range could result in inaccurate results.
  - The optimal pH range of urine specimens is between 4.6 – 8.0 and testing with specimens outside this range could result in inaccurate results.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the analytes to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the analytes with time and/or temperature may also cause false negative result as it makes analytes unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	15 months	Disposable
Detector tube	2 - 30 °C	15 months	Disposable
Detector diluent	2- 30 °C	15 months	Unopened
	2- 30 °C	3 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-167

Components of **ichroma™ AMP/EDDP/OPI**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instruction for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ AMP/EDDP/OPI**.
Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
- **i-Chamber** REF FPRR009

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ AMP/EDDP/OPI** is human urine.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- Urine specimens containing particulate materials can be centrifuged or settled to remove solid materials prior to testing.
- The sample (human urine) may be stored for two days at 2 - 8°C prior to being tested. If testing will be delayed more than two days, sample (human urine) should be frozen at -20°C.
- The sample (human urine) stored frozen at -20°C for 8 weeks showed no performance difference.
- As a repeated freeze thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ AMP/EDDP/OPI**: Sealed cartridges, detector tubes, detector diluents, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Temperature of i-Chamber should be 35°C.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.**

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the test cartridge should be 35°C during the reaction time after loading sample mixture to the test cartridge.
- To maintain the ambient temperature to 35°C, you can use various devices such as an i-chamber or an incubator and so on.

TEST PROCEDURE

▶ ichroma™ II

1) Select the sample type (Serum/Plasma/etc.) on the screen.
2) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
   (The detection buffer must be used immediately. Do not exceed a minute.)
3) Take 30 µL of sample (urine) using a pipette and dispense it to the detector tube.
4) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
   (The sample mixture must be used immediately. Do not exceed a minute.)
5) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
6) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (35 °C).
7) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 10 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
8) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
9) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
10) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
11) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ ichroma™ M3

1) Select the sample type (serum/etc.) on the screen. Press the key button to change the desired sample type.
2) Insert the cartridge approximately 1/3 into the slot of the ichroma™ M3.
   Before pushing the cartridge all the way in, ensure it is inserted in the correct orientation. An arrow is marked on the cartridge especially for this purpose.
3) The test procedure is same with the 'ichroma™ II test procedure 2) ~ 5)'.
4) Wait until the sample mixture flows into the window.
   ⚠ ***Insert the cartridge exactly 30 seconds after applying the sample. Otherwise test results may be inaccurate.***
5) Insert the sample-loaded cartridge fully into the slot of the ichroma™ M3.
6) After inserting the cartridge, the result will be automatically displayed after 10 minutes.
7) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ ichroma™ III

1) Set the temperature to 35°C by selecting test cartridge type on the screen.
2) Select the sample type (Others) on the screen.
3) The test procedure is same with 'Multi test mode 2) – 5)'.
4) Insert the sample-loaded cartridge into the holder of ichroma™ III. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
5) Tap the 'Start' button on the ichroma™ III to start the scanning process.
6) The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 10 minutes.
7) Read the test result on the display screen of ichroma™ III.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays the following results.

Test result	Display
< 1.0 x	Negative
≥1.0 x	Positive

- Cut-off (1.0 x): According to the guidelines[2)]

Analyte	Cut-off
AMP	500 ng/mL
EDDP	300 ng/mL
OPI	2,000 ng/mL

- Final diagnosis should be confirmed by another method of analysis such as GS-MS, LC-MS/MS.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- It is recommended that the control material be a third-party control material.

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  The cut-offs of AMP, EDDP, OPI are demonstrated by testing negative / cut-off ± 25% / cut-off ± 50% diluted with negative urine.

Analyte	Cut-off
AMP	500 ng/mL
EDDP	300 ng/mL
OPI	2,000 ng/mL

- **Analytical specificity**
  - **Cross-reactivity**
    Cross reaction materials listed in the following table were tested to show the concentration at positive result and cross reactivity (%). The cross-reactivity of each analyte has been calibrated against the compounds marked with an asterisk (*).

AMP	Positive result [ng/mL]	Cross-reactivity
Amphetamine*	500	100.0 %
Methamphetamine	> 1,000,000	0.0 %
3,4-Methylenedioxy methamphetamine	> 1,000,000	0.0 %

(MDMA)		
p-Methoxy amphetamine (PMA)	200	250.0 %
3,4-Methylenedioxy amphetamine (MDA)	200	250.0 %
d,l-Methyl-1 (3,4-Methylene dioxyphenyl) 2-Butanamine (MBDB)	280,000	0.2 %
p-Methoxy methamphetamine (PMMA)	150,000	0.3 %
3,4-Methylenedioxy ethylamphetamine (MDEA)	100,000	0.5 %

EDDP	Positive result [ng/mL]	Cross-reactivity
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) *	300	100.0 %
Methadone	1,200	25.0 %

OPI	Positive result [ng/mL]	Cross-reactivity
Morphine (OPI) *	2,000	100.0%
Buprenorphine	> 1,000,000	0.0 %
Codeine	2,000	100.0 %
Dihydrocodeine	8,000	25.0 %
Diacetylmorphine	8,000	25.0 %
Oxycodone	27,000	7.4 %
6-acetylmorphine	2,500	80.0 %

- **Pharmaceuticals**

Molecules such as below the ones in the table were added to the test sample(s) at concentrations, 100 µg/mL, which is much higher than their normal physiological levels in urine. **ichroma™ AMP/EDDP/OPI** test results did not show any significant cross-reactivity with these biomolecules.

Pharmaceutical		
Acetylcholine	Epinephrine	Secobarbital
Ampicillin	Erythromycin	Serotonin
Aspartame	Estradiol	Sorbitol
Atropine	Fenfluramine	Streptomycin
Barbituric acid	Fenoprofen	Sulfamethazine
Benzoic acid	Folic acid	Temazepam
Biotin	Guanidine	Testosterone
Butalbital	Hippuric acid	Tetrahydrozoline
Chloramphenicol	Inositol	Thiamine
Cholesterol	Kanamycin	Thyroxine
Clomipramine	Levothyroxine	Tocopherol
Corticosterone	Nicotine	Vitamin D
Cortisol	Penicillin G	Salsalate
Cortisone	Phenylephrine	Saponin
d,l-Octopamine	Progesterone	Tyramine
Danazol	Propranolol	Uric acid
Desipramine	Pseudoephedrine	Verapamil
Diazepam	Quinidine	Xylitol
Dopamine	Quinine	Hydroxyprogesterone
Duloxetine	Scopolamine	-

- **Interference**

Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ AMP/EDDP/OPI** test results did not show any significant interference with these materials.

Interferents	Concentration
Acetaminophen	2 mg/mL
Acetone	5 mg/mL
Acetonitrile	15 mg/mL
Acetylsalicylic acid	1 mg/mL
Ascorbic acid	20 mg/mL
Bilirubin (Conjugated)	2.5 µg/mL
Bilirubin (unconjugated)	100 µg/mL
Caffeine	0.125 mg/mL
Creatinine	2.5 mg/mL
Dextrose	10 mg/mL
Dimethyl sulfoxide	10 mg/mL
Ethanol	10 mg/mL
Gamma globulin	5 mg/mL
Hemoglobin	2 mg/mL
Human serum albumin	5 mg/mL
Ibuprofen	0.75 mg/mL
Methanol	80 mg/mL
Oxalic acid	7 mg/mL
Riboflavin	70 µg/mL
Sodium chloride	20 mg/mL
Urea	30 mg/mL
Urine specific gravity, Low (1.004)	
Urine specific gravity, High (1.040)	
Urine pH 4.5, Low	
Urine pH 8.1, High	

■ **Precision**

- **Single-site study**

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **ichroma™ AMP/EDDP/OPI** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

- **Multi-site study**

Reproducibility

1 Lot of **ichroma™ AMP/EDDP/OPI** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

AMP	Std.	Agreement/ Total	Detection ratio
Repeatability	Neg. Low	40/40	100%
	Neg. High	40/40	100%
	Pos. Low	40/40	100%
	Pos. High	40/40	100%
Total precision	Neg. Low	80/80	100%
	Neg. High	80/80	100%
	Pos. Low	80/80	100%
	Pos. High	80/80	100%

Lot to lot precision	Neg. Low	240/240	100%
	Neg. High	240/240	100%
	Pos. Low	240/240	100%
	Pos. High	240/240	100%
Reproducibility	Neg. Low	75/75	100%
	Neg. High	75/75	100%
	Pos. Low	75/75	100%
	Pos. High	75/75	100%

EDDP	Std.	Agreement/ Total	Detection ratio
Repeatability	Neg. Low	40/40	100%
	Neg. High	40/40	100%
	Pos. Low	40/40	100%
	Pos. High	40/40	100%
Total precision	Neg. Low	80/80	100%
	Neg. High	80/80	100%
	Pos. Low	80/80	100%
	Pos. High	80/80	100%
Lot to lot precision	Neg. Low	240/240	100%
	Neg. High	240/240	100%
	Pos. Low	240/240	100%
	Pos. High	240/240	100%
Reproducibility	Neg. Low	75/75	100%
	Neg. High	75/75	100%
	Pos. Low	75/75	100%
	Pos. High	75/75	100%

OPI	Std.	Agreement/ Total	Detection ratio
Repeatability	Neg. Low	40/40	100%
	Neg. High	40/40	100%
	Pos. Low	40/40	100%
	Pos. High	40/40	100%
Total precision	Neg. Low	80/80	100%
	Neg. High	80/80	100%
	Pos. Low	80/80	100%
	Pos. High	80/80	100%
Lot to lot precision	Neg. Low	240/240	100%
	Neg. High	240/240	100%
	Pos. Low	240/240	100%
	Pos. High	240/240	100%
Reproducibility	Neg. Low	75/75	100%
	Neg. High	75/75	100%
	Pos. Low	75/75	100%
	Pos. High	75/75	100%

■ **Comparability**

Performance of the **ichroma™ AMP/EDDP/OPI** kit was evaluated against a commercial reference device. A total of 205 urine samples (negative 150, positive 55) spiked with standard materials of AMP, EDDP, and OPI individually, were evaluated and results are presented in the table below.

- AMP

Total (N=205)		Reference assay	
		Positive	Negative
ichroma™ AMP/EDDP/OPI	Positive	53	2
	Negative	0	150
Sensitivity (%)		100	
Specificity (%)		98.7	
Total agreement (%)		99.0	

- EDDP

Total (N=205)		Reference assay	
		Positive	Negative
ichroma™ AMP/EDDP/OPI	Positive	50	0
	Negative	1	154
Sensitivity (%)		98.0	
Specificity (%)		100	
Total agreement (%)		99.5	

- OPI

Total (N=205)		Reference assay	
		Positive	Negative
ichroma™ AMP/EDDP/OPI	Positive	52	0
	Negative	1	152
Sensitivity (%)		98.1	
Specificity (%)		100	
Total agreement (%)		99.5	

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Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	*In vitro* diagnostic medical device
	Temperature limit
	Do not reuse
	This product fulfills the requirements of the Directive 98/79/EC on *in vitro* diagnostic medical devices

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