ichroma™ FEN/KET/MDMA

INTENDED USE

ichroma™ FEN/KET/MDMA is a fluorescence immunoassay (FIA) for the qualitative determination of Fentanyl (FEN), Ketamine (KET) and (±)-Methylenedioxymethamphetamine (MDMA) in human urine. It is useful as an aid in screening of three DOA items (FEN, KET, MDMA).

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, FEN, KET, and MDMA are the main substances that are misused and cause many social problems.[2)3)]

Fentanyl (FEN): Fentanyl is a very powerful synthetic opioid drug mainly used as a pain reliever. Fentanyl is highly addictive, and side effects caused by misuse include respiratory depression, stiffness, nausea, vomiting, constipation, dry mouth, drowsiness, confusion, and helplessness (decline).[4)]

Ketamine (KET): Ketamine is a dissociative anesthetic used medically to induce and maintain anesthesia. It is also used as an antidepressant, pain management tool, and mood-altering drug. Overdose has been known to cause ulcerative cystitis, liver toxicity, hallucinations, delirium and confusion.[5)]

(±)-Methylenedioxymethamphetamine (MDMA): MDMA, commonly known as ecstasy, is a powerful symbiotic substance with hallucinogenic effects. It is primarily abused as a party drug in places such as clubs. A side effect of long-term exposure has been reported to be nervous system toxicity.[6)]

PRINCIPLE

The test uses a competitive immunoassay method.

The antigens 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 antigens on the test strip.

More antigens 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 FEN, KET, and MDMA in the sample.

COMPONENTS

ichroma™ FEN/KET/MDMA consists of 'cartridges', 'detector tubes', and 'detector diluent'.

- The cartridge contains the membrane called a test strip which includes three carrier proteins which are conjugated with FEN, KET and MDMA individually at the test lines, and two control proteins at the control lines. 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 FEN, KET, MDMA and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The detector diluent contains tween 20 as a detergent and sodium azide as a preservative in 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 of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use 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 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 diluent 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 it 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™ FEN/KET/MDMA** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ FEN/KET/MDMA** 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.

LIMITATIONS 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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens 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
		3 months	Opened

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

MATERIALS SUPPLIED

REF CFPC-189

Components of **ichroma™ FEN/KET/MDMA**

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

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ FEN/KET/MDMA**.

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™ FEN/KET/MDMA** is human urine.

- It is recommended to test the sample within 24 hours after collection when the 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™ FEN/KET/MDMA**: Sealed cartridges, detector tubes, a detector diluent, an ID chip and instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as the 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 operation instructions.**

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 35 °C during the reaction time after loading sample mixture to the 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**

Multi test mode

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 the 'ichroma™ II test procedure 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 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 the ichroma™ III.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays four types of level results of the test sample.

Test result	Display
< 1.0 x	Negative
≥1.0 x	Positive

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

Analyte	Cut-off
FEN	5 ng/mL
KET	1,000 ng/mL
MDMA	500 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 FEN, KET, MDMA are demonstrated by testing negative / cut-off ± 25% / cut-off ± 50% diluted with negative urine.

Analyte	Cut-off
FEN	5 ng/mL
KET	1,000 ng/mL
MDMA	500 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 (*).

FEN	Positive result [ng/mL]	Cross reactivity (%)
Fentanyl*	5	100
Isobutyryl fentanyl	20	25
Norfentanyl	10	50
Ocfentanil	2,200	0.2
KET	**Positive result [ng/mL]**	**Cross reactivity (%)**
Ketamine*	1,000	100
Dextromethorphan	-	0
Phencyclidine	1,500	67

MDMA	Positive result [ng/mL]	Cross reactivity (%)
Methylenedioxymethamphetamine*	500	100
para-Methoxyamphetamine	37,000	1.4
para-Methoxy-N-methylamphetamine	20,000	2.5
3,4-Methylenedioxyamphetamine	18,000	2.8
Methylbenzodioxolylbutanamine	3,700	13.5
3,4-Methylenedioxy-N-ethylamphetamine	200	250

- **Pharmaceuticals**

Molecules listed in the following table were added to the test sample(s) at concentrations, much higher than their normal physiological levels in urine. **ichroma™ FEN/KET/MDMA** test results did not show any significant cross-reactivity with these molecules.

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™ FEN/KET/MDMA** 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™ FEN/KET/MDMA** 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™ FEN/KET/MDMA** 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.

FEN	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%

KET	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%

MDMA	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™ FEN/KET/MDMA** kit was evaluated against a commercial reference device. A total of 240 urine samples (positive 60 samples, negative 180 samples) spiked with standard materials of FEN, KET, and MDMA, individually, were evaluated and results are presented in the table below:

- **FEN**

Total (N=240)		Reference Assay	
		Positive	Negative
ichroma™ FEN/KET/MDMA	Positive	59	1
	Negative	0	178
Positive agreement (%)		100.0	
Negative agreement (%)		99.4	
Total agreement (%)		99.6	

- **KET**

Total (N=240)		Reference Assay	
		Positive	Negative
ichroma™ FEN/KET/MDMA	Positive	59	1
	Negative	0	178
Positive agreement (%)		100.0	
Negative agreement (%)		99.4	
Total agreement (%)		99.6	

- **MDMA**

Total (N=240)		Reference Assay	
		Positive	Negative
ichroma™ FEN/KET/MDMA	Positive	59	0
	Negative	1	178
Positive agreement (%)		98.3	
Negative agreement (%)		100.0	
Total agreement (%)		99.6	

REFERENCES

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2. HHS Publication, Clinical drug testing in primary care, No. (SMA) 12-4668, 2012
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4. Armenian, Patil, et al. "Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review." Neuropharmacology 134 (2018): 121-132
5. Morgan, Celia JA, H. Valerie Curran, and Independent Scientific Committee on Drugs (ISCD). "Ketamine use: a review." Addiction 107.1 (2012): 27-38
6. De la Torre, Rafael, et al. "Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition." Therapeutic drug monitoring 26.2 (2004): 137-144

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
	*In vitro* diagnostic medical device
	Temperature limit
	Do not reuse

For technical assistance, please contact:
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