MECTEST EIA by EMIT

Intended Use

MECTEST EIA by EMIT is a device intended for processing meconium into a suitable form for the analysis of cocaine or opiate by enzyme multiplied immunoassay technique (EMIT). Specifically:

a) MECTEST EIA by EMIT is a meconium processor only.

b) After processing econium, the product can be analyzed for cocaine or opiate by enzyme multiplied immunoassay technique (EMIT) using the EMIT d.a.u. Cocaine Metabolite Assay or the EMIT d.a.u. Opiate Assay (additional information on EMIT is available from Behring Diagnostics, Inc., Cupertino, CA).

Note: The MECTEST EIA by EMIT **does not contain** EMIT reagents for cocaine or opiate analysis.

c) Cut-off concentratons for cocaine and opiate in meconium by EMIT are shown:

Cocaine	25 ng/mL
Opiate	25 ng/mL

Substantial Equivalence

Substantial equivalence of MECTEST EIA by EMIT to radioimmunoassay for the analysis of cocaine and opiate in meconium are presented below (see Clinical Study). Analysis of meconium for cocaine, opiate and cannabinoid by MECTEST radioimmunoassay is an FDA cleared method (information on MECTEST radioimmunoassay is available from Mectest Corporation, Rowland Heights, CA).

Materials and Reagents Required

1. The MECTEST EIA by EMIT is a meconium processor which consists of a meconium container, a scoop and an ultrafilter. The meconium container contains a solvent, which has methanol. Avoid prolonged inhalation of vapor.
2. EMIT assays
   1) EMIT d.a.u. Cocaine Metabolite Assay
   2) EMIT d.a.u. Opiate Assay

Materials Provided

1. Meconium processor
   12 tubes, each containing meconium scoop and 5ml of solvent
   12 ultrafilters

Materials Required But Not Provided

1. EMIT d.a.u. Cocaine Metabolite Assay (available from Behring Diagnostics, Inc., Cupertino, CA)
   Antibody/substrate Reagent A
   Enzyme Reagent B
   Drug Assay Buffer
   EMIT Calibrators at 0 and 300 ng/mL
2. EMIT d.a.u. Opiate Assay (available from Behring Diagnostics, Inc., Cupertino, CA)
   Antibody/substrate Reagent A
   Enzyme Reagent B
   Drug Assay Buffer
   EMIT Calibrators Level at 0 and 300 ng/mL
3. Liquid Tox Control (0,120,350 ng/mL)
4. Meconium positive and negative controls.

Specimen Collection and Preparation

Meconium is a dark green to greenish yellow stool and is passed during the first 3 days after birth in fullterm infants or even days later in premature infants. Bright yellow stool is not meconium and should not be used for drug analysis. Meconium is a biologic specimen. Observe universal precautions, including the use of gloves, in the collection and processing of meconium to prevent the transmission of diseases, particularly, hepatitis and AIDS.

Collect meconium directly from the infant's diaper. Meconium should be mixed well in the diaper before a sample is taken since drugs are not uniformly distributed in meconium. Drug concentrations in meconium will significantly decrease if meconium is allowed to stand at room temperature for 24 hours (see below). Thus, meconium should be sampled and analyzed within 12 hours after it is excreted by the infant.

Specimen stability, transport and storage: The stability of drugs (cocaine, morphine and cannabinoid) in meconium was studied using quantitative radioimmunoassay.

1) Meconium allowed to stand at room temperature for 24 hours resulted in a 25 % decrease in cocaine. Thus, meconium should be sampled and processed as soon as possible after its excretion by the infant to prevent the loss of drugs.

2) There is no drug loss in meconium suspended in MECTEST meconium processor solvent at room temperature for at least 72 hours. Meconium should therefore be mixed well in the solvent after sampling and if left at room temperature, should be tested within 72 hours. Meconium can also be transported without refrigeration, for at least 72 hours, if suspended in MECTEST solvent.

3) For prolonged storage and drug stability, drugs are stable in meconium at -15° C for as long as 9 months.

Procedure for the Preparation & Analysis of Cocaine And Morphine in Meconium

A. Meconium Collection:

1. Mix meconium in infant's diaper before sampling. Pooling of meconium from several diapers before sampling will increase the detection rate of drugs in meconium.
2. Unscrew cap of meconium processor. Set tube in a rack (do not spill reagent). Use scoop attached to the cap to obtain 0.5 g of meconium (approximately one scoopful of meconium).
3. Place cap and scoop containing meconium back into meconium processor.
4. Screw cap tightly.

Note: If meconium is sent to an outside laboratory for analysis, meconium should be vortexed and emulsified in meconium solvent (see meconium processing, step 1) prior to mailing.

B. Meconium Processing

1. Vortex meconium processor using scoop as a stirrer until meconium is evenly dispersed in the solvent. (To test, lay tube on its side – globs of meconium should not be seen).
2. Detach scoop from cap and discard scoop.
3. Recap tube and centrifuge at 4600 rpm (3000 g) for 30 minutes.
4. Collect supernate and transfer 1 mL into the ultrafilter provided in the kit. (Save remaining supernate for future use).
5. Centrifuge at 4600 rpm (3000 g) for 30 minutes. Let stand for 10 minutes to avoid overheating. Centrifuge at 4600 rpm for another 30 minutes.
6. Collect total ultrafiltrate (approximately 250 microliters) for drug analysis.

C. Preparation of Standards and Meconium Controls

1. Standards and controls for cocaine analysis by EMIT
   a) Prepare benzoylecgonine standards at 0, 25 and 300 ng/mL. Dilute EMIT calibrators with buffer for desired concentrations.
   b) Tox Control at 0, 120 and 350 ng/mL (available from Medical Analysis System, Camarillo, CA).
   c) Meconium controls
      1) Negative meconium control: Obtain 0.5 g of drug free meconium. Process meconium as above
      2) Positive meconium control (25 ng/mL): Obtain 0.5 g of drug free meconium. Add 0.5 mL of 300 ng/mL EMIT Calibrator. Process meconium as above.

2. Standards and controls for opiate analysis by EMIT
   a) Prepare morphine standards at 0, 25 and 300 ng/mL. Dilute EMIT calibrators with buffer for the desired concentrations.
   b) Tox Control at 0, 120 and 350 ng/mL (available from Medical Analysis System, Camarillo, CA).
   c) Meconium controls
      1) Negative meconium control: Obtain 0.5 g of drug free meconium. Process meconium as above.
      2) Positive meconium control (27 ng/mL): Obtain 0.5 g of drug free meconium. Add 0.5 mL of 300 ng/mL EMIT Calibrator. Process meconium as above.

D. Procedure for Meconium Analysis by EMIT

1. Set drug standards for calibration.
2. Use 25 microliters of Tox Control and ultrafiltrates from meconium control and patient meconium for each drug analysis.
3. Analyze meconium ultrafiltrate and controls for cocaine and opiate by EMIT (additional information on EMIT assay is available from Behring Diagnostics, Inc., Cupertino, CA).

Reporting of Results

Positive Results

The cut-off concentrations for cocaine or opiate by EMIT are shown in the table below. A Sample that gives a change in absorbance (AA) value equal to or higher than the AA value of the 25 ng/mL standard is interpreted as a preliminary positive result.

Note: A positive test is a preliminary result only and should be confirmed by a more specific method. Gas chromatography /mass spectrometry (GC/MS) is the preferred method of confirmation.

Cut-off concentrations for cocaine and opiate by EMIT

Cocaine	25 ng.mL
Opiate	25 ng/mL

Negative Results

The cut-off concentrations for cocaine and opiate by EMIT are shown in the table below. A sample that gives a change in absorbance (AA) value lower than the AA value of the 25 ng/mL standard is interpreted as negative.

Cut-off concentrations for cocaine and opiate by EMIT

Cocaine	25 ng/mL
Opiate	25 ng/mL

Warning: A negative result does not eliminate the possibility of drugs of abuse consumption by the mother. When meconium is used in any other system/method for the detection of drugs of abuse, the user must be aware that the concentration of the drug of abuse in meconium has not been correlated with the dose of the drug of abuse consumed by the mother, nor with the clinical picture in the neonate. The performance characteristics of other systems/methods have not been determined by the manufacturer nor have been FDA cleared.

Performance Characteristics

A. Sensitivity

1. **Enzyme multiplied immunoassay technique (EMIT)**

a) *Cocaine*. The sensitivity of the MECTEST EIA by EMIT for cocaine was determined by spiking meconium with different amounts of benzoylecgonine and analyzing the different concentrations by EMIT: 0.5 g of drug free meconium was suspended in 5 mL of solvent in the MECTEST processor. The mixture was vortexed for 5 minutes. Known amounts of benzoylecgonine were added to the meconium suspension to achieve concentrations ranging from 0 to 100 ng/mL. A positive test was seen at benzoylecgonine concentration of 25 ng/mL (see table). Since EMIT is a qualitative test, a benzoylecgonine (BE concentration in meconium of at least25 ng/mL is recommended as the cut-off concentration for a positive test.

BE concentration (ng/mL)	EMIT
0	-
0	-
0	-
25	+
25	+
50	+
50	+
100	+
100	+

b) Morphine. The sensitivity of the MECTEST EIA by EMIT for opiate was determined by spiking meconium with different amounts of morphine and analyzing the different concentrations by EMIT: 0.5 g of drug free meconium was suspended in 5 mL of solvent in the MECTEST processor. The mixture was vortexed for 5 minutes. Known amounts of morphine were added to the meconium suspension to achieve concentrations ranging from 0 to 100 ng/mL. As shown in the table below, a positive EMIT test was seen at morphine concentration of 25 ng/mL. Since EMIT is a qualitative test, a morphine concentrations in meconium of at least 25 ng/mL is recommended as the cut-off concentration for a positive test.

Morphine concentration (ng/mL)	EMIT
0	-
0	-
0	-
25	+
25	+
50	+
50	+
100	+
100	+

B. Specificity

1. **Enzyme multiplied immunoassay technique (EMIT).** Meconium was found to contain endogenous amounts of bilirubin (60.23 $\pm$ 2.76 micrograms/gm meconium), blood (0 to +++ hemoglobin by qualitative guaiac test) and protein (32.89 $\pm$ 12.36 mg/gm meconium) which did not interfere in the detection of benzoylecgonine or morphine in meconium. In meconium spiked with cocaine, opiate and cannabinoid, cross reaction was not seen between the drugs when analyzed by EMIT and radioimmunoassay (RIA).

Drug concentration (ng/mL)					Morphine detection by	
Morphine	Cocaine	Cann	RIA	EMIT	RIA	EMIT
100	100	100	+	+	+	+
100	100	0	+	+	+	+
100	0	100	-	-	+	+
0	100	100	+	+	-	-
200	200	200	+	+	+	+
200	200	0	+	+	+	+
200	0	200	-	-	+	+
0	200	200	+	+	-	-

C. Accuracy

1. **Enzyme multiplied immunoassay technique (EMIT**)

a) *Interassay precision.* Determination of drug concentrations of 6 meconium samples, each spiked either with 50 or 100 ng/mL of cocaine and morphine showed a mean coefficent of variability of 2.35 $\pm$ 2.19% for cocaine and 1.5 $\pm$ 1.27% for morphine

b) *Intra-assay precision.* Triplicate analysis for cocaine and morphine were done on 4 meconium samples. A coefficient of variability was obtained for each triplicate analysis. The mean (sd) coefficient of variability for the 4 samples was 0.52 $\pm$ 0.38% for cocaine and 0.41 $\pm$ 0.28% for morphine.

D. Clinical Study

1. **Enzyme multiplied immunoassay technique (EMIT).** For substantial equivalence, enzyme immunoassay by EMIT was compared to radioimmunoassay in 61 samples. The study was supplemented with 20 additional samples in which EMIT was compared to GC/MS.

a) *Cocaine.* Meconium was obtained from 61 infants and analyzed for cocaine by EMIT and RIA. The concordance of positive and negative results were 95% and 98% respectively.

EMIT	Radioimmunoassay		
	Negative	Positive	Total
Negative	21	1	22
Positive	1	38	39
Total	22	39	61

Concordance of positive results = 38/39 (98%);
Concordance of negative results = 21/22 (95%)

Meconium was obtained from 20 infants and analyzed for cocaine by EMIT and GC/MS. The following results show 100% concordance of positive and negative tests by the 2 methods.

No	EMIT	GCMS	No	EMIT	GCMS
1	+	+	11	+	+
2	+	+	12	+	+
3	+	+	13	+	+
4	+	+	14	+	+
5	+	+	15	-	-
6	+	+	16	-	-
7	+	+	17	+	+
8	+	+	18	+	+
9	+	+	19	+	+
10	+	+	20	+	+

a) *Opiate*. Meconium was obtained from 61 infants and analyzed for morphine by EMIT and RIA. The concordance of positive and negative results were 100% and 98%. respectively.

EMIT	Radioimmunoassay		
	Negative	Positive	Total
Negative	52	0	52
Positive	1	8	9
Total	53	8	61

Concordance of positive results = 8/8 (100%);
Concordance of negative results = 52/53 (98%)

No.	EMIT	GCMS	No.	EMIT	GCMS
1	+	+	11	+	+
2	+	+	12	+	+
3	+	+	13	+	+
4	+	+	14	+	+
5	+	+	15	-	-
6	+	+	16	+	+
7	+	+	17	+	+
8	+	+	18	+	+
9	+	+	19	+	+
10	-	-	20	+	+

E. Effect of Carryover

Patient samples may occasionally have very high concentrations of cocaine or morphine. It is suggested that routine precautions be taken, e.g., employing a fresh pipet tip for each sample, to avoid carry-over contamination.

F. Limitations: Based on a review of the literature, the following may cause false positive reactions:
1. Maternal medications containing morphine, codeine or other opioids
2. Maternal ingestion of food or drinks containing poppy products or coca products (such as herbal tea).
3. Passive maternal inhalation of cocaine or opiate.
4. Technical or procedural errors.

G. Quality Control
1. *Record keeping*. It is good laboratory practice to record for each assay the lot numbers and reconstitution dates of the components used.
2. *Sample handling* It is good laboratory policy to maintain accurate chain of custody of specimens. The instructions for the proper collection, handling and storage of samples should be followed. Criteria for non acceptance of specimen, include: a) improperly identified specimen, b) leakage of specimen container, c) broken container. The instructions for handling and storing patient samples and components should be carefully observed. Dilute high patient samples with the kit's zero calibrator prior to assay. All samples, including the calibrators and controls, should be assayed in duplicate. It is good laboratory practice to use a disposable-tip micropipet, changing the tip between samples, in order to avoid carry-over contamination.
3. *Controls*. We recommend that in accordance with guidelines set by the National Institute on Drug Abuse, controls should be assayed at or near the cutoff concentrations and the results charted from day to day.

H. Clinical Application

The analysis of drugs and their metabolites in meconium is a new and sensitive method for identifying infants who have been exposed to drugs in utero .[1-2] Meconium represents the first series of green stools of the newborn infant which are passed within a few days after birth. The concept behind meconium testing was based on initial research in animals which showed that a high concentration of the drugs which the pregnant animal was exposed to, were present in the meconium of their fetuses. [2-5] Drugs which the fetus is exposed to during pregnancy are metabolized by its liver into water soluble metabolites and excreted into the bile or urine. It is postulated that drug deposition in meconium occurs either through bile secretion or through swallowing by the fetus of its urine via the amniotic fluid. Clinical studies in humans have validated meconium analysis as a sensitive drug screen in the newborn infant. [6-9] The initial clinical study compared drug detection in 20 infants of drug dependent mothers by meconium and urine analysis.[1] Whereas all meconium samples contained either cocaine, opiate or cannabinoid, only 37% of the urine tested was positive for these drugs. Subsequent studies have corroborated the sensitivity of meconium drug testing. In one study, meconium was analyzed for cocaine, morphine, codeine and marijuana from 28 neonates born to women suspected of drug abuse. [9] In each case, testing of urine from the mother, the infant or both were done because of suspected maternal drug abuse. Compared with the combination of maternal and newborn urine testing, meconium testing had an 82% positive predictive value and a 91% negative predictive value. The authors further added that the collection of meconium was simpler and more reliable than collection of urine and that the testing of meconium was easily incorporated into routine procedures at a busy commercial laboratory. In another study, a comparison of the sensitivity of meconium and urine analyses for drugs in detecting gestational exposure to cocaine was studied. [8] The infants were born to 59 women who were interviewed to determine their use of cocaine during pregnancy. Radioimmunoassay and gas chromatography of meconium were more sensitive than immunoassay of urine ($p<0.02$). Urine immunoassay failed to identify 60% of cocaine exposed infants. The largest clinical study using meconium drug testing was a drug prevalence study conducted in a large, high risk, obstetric population.

The superiority of meconium testing over maternal history was demonstrated. A fourfold (44.3% vs 11.1%) higher incidence of drug exposure was found among 3010 infants tested by meconium analysis as compared to maternal history. The meconium drug test has also been adapted for mass drug screening of newborn infants [10] and selection criteria for routine testing of infants have been formulated.

Recently, other studies have been published illustrating the clinical application of the meconium drug test. The meconium test was used to prospectively screen for drugs (opiates, cocaine and cannabinoids) every infant who was admitted to the neonatal intensive care unit of a high risk perinatal center for a 3 month period. [12] Of the 82 infants tested, 41 or 50% were positive for drugs: 36 (44%) positive for cocaine, 9 (11%) positive for opiates and none for cannabinoid. The total cost for the care of these infants was $1,223,750. The authors concluded that there is a high prevalence of drug exposure in infants admitted to the neonatal intensive care and that the morbidity, mortality and medical cost, associated with drugs, are significant. A biologic marker of fetal exposure to nicotine in passive and active maternal smoking has also been determined by meconium analysis. [13] Nicotine metabolites (cotinine and trans 3'- hydroxycotinine) were detected in meconium at concentrations proportional to the degree of maternal active and passive smoking. Furthermore, in utero exposure to tobacco smoke in infants of passive smokers was as high as among infants whose mothers actively smoked less than 1 pack per day during pregnancy. Lastly, a comparative methodologic study was done to detect in utero cocaine exposure in infants. Maternal history was compared with various assays in meconium, Maternal urine and infant's urine, using GC/MS, EMIT, ADx and DPC radioimmunoassay. The authors found meconium to be superior to either maternal or infant urine in detecting in utero cocaine exposure, although the need for concomitant maternal histories in some cases was emphasized.

In summary, meconium drug testing is ideal in the newborn period for several reasons: (i) the test is highly sensitive and specific, (ii) the test can be performed using common laboratory techniques for purposes of mass screening and with capabilities for GC/MS confirmation, (iii) collection of meconium is easy and non invasive, (iv) analysis of serial meconium can reflect the type, chronology and amount of in utero drug exposure of the infant is and, (v) drugs in meconium are present up to the third day after birth; thus late testing of the infant for drugs is possible. Meconium drug testing has therefore become a useful tool for clinical and research needs.

References

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