MECTEST PROCESSOR

Intended use

MECTEST PROCESSOR is a device intended for processing meconium into a suitable form for the analysis of drugs of abuse using FDA-cleared methods: radioimmunoassay (RIA), enzyme multiplied immunoassay technique (EMIT) and fluorescence polarization immunoassay (FPIA). Specifically:

a) MECTEST PROCESSOR is a meconium processor only.

b) After processing meconium, the product (analyte) can be subjected to analysis for cocaine, opiate and cannabinoid by radioimmunoassay using MECTEST Cocaine RIA, MECTEST Opiate RIA and MECTEST Cannabinoid RIA (Additional information on the MECTEST RIA kits are available upon request from Mectest Corporation, Rowland Heights, CA.

c) After processing meconium, the product (analyte) can be subjected to analysis for cocaine and opiate by enzyme multiplied immunoassay technique (EMIT) using EMIT d.a.u. Cocaine Metabolite Assay and the EMIT d.a.u. Opiate Assay (Additional information for EMIT analysis is available from Behring Diagnostics, Inc, Cupertino, CA)

d) After processing meconium, the product (analyte) can be subjected to analysis for cocaine and opiate by fluorescence polarization immunoassay (FPIA) using ADX FPIA Cocaine Metabolite Assay System and the ADX FPIA Opiate Assay System (Additional information for FPIA analysis is available from Abbott Laboratories, Abbott Park, IL)

The cut off concentrations for cocaine, opiate and cannabinoid by radioimmunoassay, EMIT and FPIA are shown:

	RIA (ng/mL)	EMIT (ng/mL)	FPIA (ng/mL)
Cocaine	7.62	25	25
Opiate	27.82	25	25
Cannabinoid	6.54	-	-

Background of Meconium Drug Testing

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.[2] 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 (14/17) 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.[6] 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. [11]

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 [15] 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.

Ordering Information

To order, call (626) 674-7532. Please have your Customer Number ready, to facilitate the processing of your order. All sales must be considered final, as the products cannot be returned to stock, once having left the manufacturer's direct control. Kit and component prices, quotations for standing orders and quantity discounts will be furnished on request. Shipping is at ambient temperature, F.O.B. the site of manufacture in Rowland Heights, CA.

A copy of the customer's Radioisotope License must be on file with Mectest Corporation before kits or components containing radioactive material can be shipped. These may be acquired only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories and hospitals - and strictly for in vitro clinical or laboratory tests not involving external or internal administration of the radioactive material or its radiation to human beings or other animals. Its acquisition, receipt, storage, use, transfer and disposal are all subject to the regulations and a (general or specific) license of the U.S.Nuclear Regulatory Commission or of a State with which the N.R-C. has entered into an agreement for the exercise of regulatory control.

Principle of MECTEST. Meconium is emulsified in buffered methanol at a specific pH for the extraction of drugs and their metabolites. The suspension is centrifuged and the supernate is ultrafiltered. The protein free ultrafiltrate is analyzed for cocaine, opiates and cannabinoid by radioimmunoassay (RIA) or enzyme immunoassay by enzyme multiplied immunoassay technique (EMIT) or by fluorescence polarization immunoassay (FPIA).

MECTEST Processor

The MECTEST Processor contains 12 meconium processing tubes with solvent and 12 ultrafilters.

Reagents. The MECTEST processor contains 5 cc of buffered methanol. The MECTEST processor is included in all MECTEST radioimmunoassay kits, e.g.., MECTEST Cocaine Radioimmunoassay , MECTEST Opiate Radioimmunoassay and MECTEST Cannabinoid Radioimmunoassay . The MECTEST EIA by EMIT and FPIA **only** contains the MECTEST processor; it does not contain the reagents for EMIT or FPIA analysis .

MECTEST is a proprietary product and is protected by U.S. Patent Nos. 5,015,589 and 5,185,267. MECTEST is an in vitro diagnostic device for human use.

Safety. Contains methanol. Avoid prolonged inhalation of vapor. For in vitro diagnostic use only.

Storage. Store at room temperature. Keep tubes covered in container when not in use. Protect from excessive light and heat. Reagent is stable for at least one year from the date of manufacture. Check expiration date on the box. Before use, check that solution in tube is clear and colorless. Do not use, if solution is turbid or discolored. Discard tube after use. Do not reuse.

General Principle. Meconium represents stools of the infant which were formed during gestation. It is dark green to greenish yellow stool and is passed during the first 3 days after birth in fullterm infants or many 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 acquired immunodeficiency syndrome or AIDS.

Meconium is collected 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 decrease if meconium is allowed to stand at room temperature for 24 hours (see below - Drug Stability). Thus, meconium should be sampled from the diaper, as soon as possible, after it is excreted by the infant.

At room temperature, there is no drug loss in meconium for at least 72 hours, if meconium is suspended in MECTEST solvent. (see below- Drug Stability). Thus, meconium can also be transported, without refrigeration for at least 72 hours, if suspended in MECTEST solvent. For drug stability during prolonged storage, drugs are stable in meconium for at least 9 months if meconium is frozen at -15^{o} C.

Procedure for the Preparation and Analysis of Drugs of Abuse in Meconium

A. Meconium Collection

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

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

B. Meconium Processing

1. Vortex MECTEST processor, with scoop acting as a stirrer, until meconium is evenly dispersed in the solvent. (To test, lay tube at 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 min. Let stand for 10 min. Centrifuge again at 4600 rpm x 30 min.
6. Collect ultrafiltrate (approximately 250 microliter) for drug analysis.

C. Meconium Drug Analysis

1. Use 25 microliters of ultrafiltrate for each drug analyzed.
2. Analyze ultrafiltrate for cocaine, opiate and cannabinoid by radioimmunoassay using MECTEST Cocaine Radioimmunoassay, MECTEST Opiate Radioimmunoassay and MECTEST Cannabinoid Radioimmunoassay.
3. Analyze ultrafiltrate for cocaine and opiate by EMIT (see MECTEST EIA by EMIT for opiate or cocaine analysis).
4. Analyze ultrafiltrate for cocaine and opiate by FPIA (see MECTEST EIA by FPIA for opiate or cocaine analysis).

D. Reporting of Results

1) *Negative test.* Drug concentrations in meconium, which are below the cut off concentrations, shown below, are reported as "negative" for the drug/s tested

Cut off concentrations of drugs by RIA, EMIT and FPIA

	RIA (ng/mL)	EMIT (ng/mL)	FPIA (ng/mL)
Cocaine	7.62	25	25
Opiate	27.82	25	25
Cannabinoid	6.54	-	-

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.

2) *Positive test.* Drug concentrations in meconium, which are at or greater than the cut off concentrations, shown below, are reported as "presumptive positive" for the drug/s tested.

Cut off concentrations of drugs by RIA, EMIT and FPIA

	RIA (ng/mL)	EMIT (ng/mL)	FPIA (ng/mL)
Cocaine	7.62	25	25
Opiate	27.82	25	25
Cannabinoid	6.54	-	-

Warning: These are only preliminary test results. All positive tests should be confirmed by more specific methods. Gas chromatography/mass spectrometry (GC/MS) is the confirmatory method of choice.

Performance Characteristics

A. Sensitivity

1. **Radioimmunoassay (RIA)**

a). *Cocaine*. The sensitivity of the MECTEST Cocaine Radioimmunoassay was determined by spiking meconium with different amounts of benzoylecgonine and analyzing the benzoylecgonine concentrations by RIA. A stock solution (10,000 ng/mL) of the benzoylecgonine was prepared in methanol. The concentration of the stock solution was analyzed by RIA and the amount of drug spiked into the meconium suspension was calculated based on the analyzed concentration of the stock solution. Spiked meconium was prepared as follows: 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 from the stock solution were added to the meconium suspension to achieve 11 drug concentration levels ranging from 0 to 207 ng/mL. The spiked meconium was processed using MECTEST and the ultra filtrate was analyzed for cocaine (observed concentration) by MECTEST Cocaine Radioimmunoassay.

Results: The observed and expected concentrations of cocaine (benzoylecgonine) in meconium were plotted in a linear regression model which showed the following results: correlation coefficient (r) = 0.988 ($p<0.0001$), goodness of fit (r^2) = 0.976, constant = -4.72 ng/mL and slope of the regression line = 0.988. The minimum detectable (cutoff) concentration for cocaine = 7.62 ng/mL (derived from the intercept of the regression line at the 95% confidence limit).

b) *Morphine*. The sensitivity of MECTEST Opiate Radioimmunoassay was determined by spiking meconium with different amounts of morphine and analyzing the morphine concentrations by RIA. A stock solution (10,000 ng/mL) of morphine 3 glucuronide was prepared in methanol. The concentration of the morphine stock solution was analyzed by RIA and the amount of each drug spiked into the meconium suspension was calculated based on the analyzed concentration of the stock solution. The spiked meconium was prepared as follows: 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 3 glucuronide standards were added to meconium to achieve 13 drug concentration levels ranging from 0 to 423 ng/mL. The ultrafiltrate was analyzed for morphine (observed concentration) by MECTEST Opiate Radioimmunoassay.

Results: The observed and expected concentrations of morphine 3 glucuronide in meconium were plotted in a linear regression model which showed the following results: correlation coefficient (r) =0.994 ($p<0.0001$), goodness of fit (r^2) = 0.989, constant =14.95 ng/mL and slope of the regression line =0.697. The minimum detectable (cutoff) concentration for morphine = 27.82 ng/mL (derived from the intercept at the 95% confidence limit of the regression line).

c). *Cannabinoid*. The sensitivity of MECTEST Cannabinoid Radioimmunoassay was determined by spiking meconium with different amounts of 11-nor, delta 9 tetrahydro-cannabinol-9-carboxylic acid and determining the different cannabinoid concentrations by RIA. A stock solution (10,000 ng/mL) of 11-nor, delta 9 tetrahydro- cannabinol-9-carboxylic acid was prepared in methanol. The concentration of the stock solution was analyzed by RIA and the amount of cannabinoid spiked into the meconium suspension was calculated based on the analyzed concentration of the stock solution. The spiked meconium was prepared as follows: 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 11-nor, delta-9-tetrahydrocannabinol-9-carboxylic acid from the stock solution were added to meconium to achieve 10 drug concentration levels ranging from 0 to 127 ng/mL. The ultrafiltrate was analyzed for cannabinoid (observed concentration) by the MECTEST Cannabinoid Radioimmunoassay.

Results: The observed and expected concentrations of 11-nor, delta-9-tetrahydro cannabinol-9-carboxylic acid in meconium were plotted in a linear regression model which showed the following results:

correlation coefficient (r) = 0.994 ($p<0.0001$), goodness of fit (r^2) = 0.988, constant = 0.940 ng/mL and slope of the regression line = 1.33 ng/ mL . The minimum detectable (cutoff) concentration for cannabinoid = 6.55 ng/mL (derived from the intercept of the regression line at the 95% confidence limit).

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

a) *Cocaine.* The sensitivity of the Mectest EIA for cocaine by EMIT 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) concentrations in meconium of at least 25 ng/mL is recommended as 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 for opiate by EMIT 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	+

3. **Fluorescence polarization immunoassay (FPIA)**

a) *Cocaine*. The sensitivity of the Mectest EIA for cocaine by FPIA was determined by spiking meconium with different amounts of benzoylecgonine (BE) and analyzing the different concentrations by radioimmunoassay and FPIA: 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 FPIA test was seen at benzoylecgonine concentration of 25 ng/mL as shown in table below. Since FPIA is a qualitative test, a benzoylecgonine concentration in meconium of at least 25 ng/mL is recommended as the cut-off concentration for a positive test.

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

b) *Morphine.* The sensitivity of the Mectest EIA for morphine by FPIA was determined by spiking meconium with different amounts of morphine and analyzing the different concentrations by FPIA: 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. A positive FPIA test was seen at morphine concentration of 25 ng/mL. Since FPIA 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)	FPIA
0	-
0	-
0	-
25	+
25	+
50	+
50	+
100	+
100	+

B. Specificity

1. **Radioimmunoassay.** Meconium was found to contain endogenous amounts of bilirubin (60.23 ± 2.76 micrograms/gm meconium), blood (0 to +++ hemoglobin by qualitative guaiac test) and protein (32.89 ± 12.36 mg/gm meconium) which did not interfere in the recovery of cocaine, opiate or cannabinoid in meconium. Recovery rate was 104.5 ± 14.1% for cocaine, 135 ± 9.6% for morphine and 96.6 ± 6.2% for cannabinoid. No cross reaction was observed between cocaine, opiate or cannabinoid at the concentrations used. There was 0% to 0.2% cross reactivity of cocaine, morphine or cannabinoid to the following drugs which were prepared at concentrations of 100,000 ng/mL: acetaminophen, phenobarbital, acetylsalicylic acid,, propoxyphene, pentazocine, chlorpromazine, ibuprofen, meperidine, diazepam, lidocaine and caffeine.

2. **Enzyme multiplied immunoassay technique (EMIT)** Meconium was found to contain endogenous amounts of bilirubin (60.23 ± 2.76 micrograms/gm meconium), blood (0 to +++ hemoglobin by qualitative guaiac test) and protein (32.89 ± 12.36 mg/gm meconium) which did not interfere in the detection of benzoylecgonine or morphine in meconium by EMIT. 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)			Cocaine Detection by		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	+	+	-	-

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

Drug concentration (ng/mL)			Cocaine detection by		Morphine detection by	
Morphine	Cocaine	Cann	RIA	FPIA	RIA	FPIA
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. **Radioimmunoassay**

a) *Interassay precision.* Known amounts of cocaine, morphine and cannabinoid were spiked into 5 meconium samples (0.5 g per sample) to give a drug concentration in each sample of 200 ng/mL. Each sample was individually processed using the MECTEST processor and analyzed for cocaine, morphine and cannabinoid by radioimmunoassay. The interassay coefficient of variability (CV) was 8.327% for cocaine, 1.053% for morphine and 8.91% for cannabinoid.

b) *Intra-assay precision*. Triplicate analysis for cocaine, morphine and cannabinoid were done on 8 meconium samples. A coefficient of variability was obtained for each triplicate analysis and a mean coefficient of variability for the 8 samples was calculated. The mean (sd) coefficient of variability was 5.9 $\pm$ 3.9% for cocaine, 4.5 $\pm$ 1.3% for morphine and 5.3 $\pm$ 2.6 % for cannabinoid.

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

a) *Interassay precision.* Determination of drug concentrations in 6 meconium samples, each spiked with either 50 or 100 ng/mL of cocaine and morphine showed a mean coefficient 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.

3. **Fluorescence polarization immunoassay (FPIA).**

a) *Interassay precision.* Determination of drug concentrations in 6 meconium samples, each spiked with either 50 or 100 ng/mL of cocaine and morphine showed mean coefficient of variability of 0.78 $\pm$ 0.11% for cocaine and 0.44 $\pm$ 0.02% 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 was 0.34 $\pm$ 0.19% for cocaine and 0.43 $\pm$ 0.11% for morphine.

D. Drug Stability in Meconium.

The stability of cocaine, morphine and cannabinoid in meconium were tested under the following conditions: (1) at room temperature for 24 hours, (2) meconium, emulsified in MECTEST solvent for 72 hours at room temperature and (3) at -15^{o} C for at least 9 months. Quantitative determination of drug concentrations was done by radioimmunoassay.

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

Mectest Processor

	0 hr (ng/mL)	24 hr (ng/mL)	% change
Cocaine			
Sample 1	518.88	350.24	-32.5
Sample 2	337.73	270.43	-19.9
Sample 3	638.42	463.51	-27.4
Sample 4	561.76	378.17	-32.7
Morphine			
Sample 1	160.61	218.36	+36
Sample 2	119.44	197.83	+65.6
Sample 3	321.75	599.11	+86.2
Sample 4	285.03	459.80	+61.3

Mectest Processor

Cannabinoid			
Sample 1	46.0	98.8	+114
Sample 2	40.30	36.83	-8.6
Sample 3	149.36	69.72	-53.3
Sample 4	76.86	54.31	-29.3

2) Meconium emulsified in MECTEST solvent and kept at room temperature for 72 hours did not show a decrease in its concentration of cocaine, morphine or cannabinoid (see below). Thus, there is no drug loss in meconium suspended in MECTEST solvent at room temperature for at least 72 hours. The increase in morphine concentration was due to the hydrolysis of morphine glucuronide to morphine due to the presence of beta glucuronidase in meconium

	0 hr (ng/mL)	72 hr (ng/mL)	% change
Cocaine			
Sample 1	518.88	527.12	+1.0
Sample 2	337.73	353.73	+4.7
Sample 3	638.42	384.01	+7.1
Sample 4	561.76	618.81	+10.2
Morphine			
Sample 1	160.61	585.93	+264.8
Sample 2	119.44	299.07	+150.4
Sample 3	321.75	1200.57	+273.1
Sample 4	285.03	1145.79	+302.0
Cannabinoid			
Sample 1	46.0	51.46	+11.9
Sample 2	40.30	33.41	-17.1
Sample 3	149.36	194.10	+30.0
Sample 4	76.86	82.26	+7.0

3. Drugs are stable in meconium at -15 degrees C for at least 9 months

Days Bet-wee tests	Cocaine (ng/mL)		Opiate (ng/mL)		Cann (ng/mL)	
	Initial conc	Last conc	Initial conc	Last conc	Init conc	Last conc
322	13611	16809	1225	1217	0	0
322	16122	21249	1242	1100	0	0
322	2683	6246	156	252	0	0
170	6629	9472	144	113	0	0
170	10223	9608	823	545	77	42
170	20605	16952	2368	2149	101	96

170	1475	2286	0	0	297	278
109	3410	2802	0	0	0	0
120	19581	17903	4479	2996	0	0
109	16566	19881	0	0	0	0
109	21783	18612	0	0	0	0

E. Clinical Study

1. **Radioimmunoassay.** For substantial equivalence, RIA was compared to GC/MS in 50 meconium samples analyzed for cocaine, opiate and cannabinoid.

a) *Cocaine.* 50 meconium samples from in utero drug exposed and control infants were analyzed for cocaine by MECTEST Cocaine Radioimmunoassay and gas chromatography/ mass spectrometry (GC/MS)[15]. The table shows 100% agreement between the RIA and GC/MS results.

No.	RIA	GCMS	No.	RIA	GCMS
1	16809	+	26	299	+
2	45.29	+	27	6429	+
3	27.88	+	28	5568	+
4	9472	+	29	1342	+
5	9608	+	30	96	+
6	25.526	+	31	0	-
7	77.77	+	32	1229	+
8	2802	+	33	1927	+
9	17903	+	34	1507	+
10	19881	+	35	7474	+
11	18612	+	36	3409	+
12	13487	+	37	3058	+
13	2113	+	38	0	-
14	1166	+	39	0	-
15	0	-	40	3426	+
16	0	-	41	10222	+
17	32920	+	42	51	+
18	25403	+	43	0	-
19	42836	+	44	0	-
20	43143	+	45	0	-
21	28171	+	46	16122	+
22	22909	+	47	347	+
23	21303	+	48	5753	+
24	8699	+	49	0	-
25	0	-	50	16565	+

Mectest Processor

b) *Opiate.* 50 meconium samples from in utero drug exposed and control infants were analyzed for opiate by MECTEST radioimmunoassay and gas chromatography/mass spectrometry (GC/MS) [15].

The table shows agreement between the RIA and GC/MS results.

No.	RIA	GCMS	No.	RIA	GCMS
1	1217	+	26	2379	+
2	1100	+	27	2561	+
3	252	+	28	0	-
4	113	+	29	35	+
5	545	+	30	1838	+
6	2149	+	31	0	-
7	0	-	32	3517	+
8	0	-	33	747	+
9	2996	+	34	4507	+
10	0	-	35	2252	+
11	0	-	36	779	+
12	0	-	37	360	+
13	1507	+	38	958	+
14	1195	+	39	1946	+
15	0	-	40	1231	+
16	829	+	41	3074	+
17	217	+	42	1026	+
18	2379	+	43	0	-
19	137	+	44	1390	+
20	581	+	45	608	+
21	1646	+	46	233	+
22	118	+	47	0	-
23	156	+	48	822	+
24	0	-	49	2367	+
25	1881	+	50	0	-

Morphine was the most common opiate identified by GC/MS; however, in some samples (#3, 4, 25, 29 and 33), the opiate was codeine. Since

RIA detects opiate as a group, GC/MS is necessary to identify the specific opiate

c) *Cannabinoid.* 50 meconium samples from in utero drug exposed and control infants were analyzed for cannabinoid by radioimmunoassay

Analysis of cannabinoid by RIA and GC/MS

No.	RIA	GCMS	No.	RIA	GCMS
1	9	+	26	31.15	+
2	0	-	27	0	-
3	282	+	28	0	-
4	231.10	+	29	0	-
5	56.28	+	30	0	-
6	96	+	31	8.02	+
7	278	+	32	0	-
8	30.71	+	33	61	+
9	144.2	+	34	86	+
10	0	-	35	0	-
11	151.78	+	36	0	-
12	0	-	37	13	+
13	54.19	+	38	0	-
14	56.8	+	39	0	-
15	0	-	40	0	-
16	61.9	+	41	17	+
17	86.4	+	42	0	-
18	13	+	43	42	+
19	0	-	44	0	-
20	0	-	45	0	-
21	0	-	46	0	-
22	0	-	47	0	-
23	0	-	48	0	-
24	20.59	+	49	0	-
25	49.52	+	50	0	-

2. **Enzyme multiplied immunoassay technique (EMIT).** For substantial equivalence, enzyme immunoassay by EMIT was compared to RIA 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 were95% 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%)

2) 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	+	+

b) *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%)

Meconium was obtained from 20 infants and analyzed for morphine by EMIT and GC/MS. There was high concordance of positive and negative tests between the 2 tests as shown in the following table.

No.	EMIT	GCMS	No.	EMIT	GCMS
1	+	+	11	+	+
2	+	+	12	+	+
3	+	+	13	+	+
4	+	+	14	+	+

and GC/MS [15]. The table shows agreement between the RIA and GC/MS results.

5	+	+	15	-	-
6	+	+	16	+	+
7	+	+	17	+	+
8	+	+	18	+	+
9	+	+	19	+	+
10	-	-	20	+	+

3. **Fluorescence polarization immunoassay (FPIA)**. For substantial equivalence, FPIA was compared to RIA in 61 samples and in 20 additional samples, FPIA was also compared to GC/MS.

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

FPIA	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 FPIA and GC/MS. The following results show 100% concordance of positive and negative tests by the 2 methods.

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

b) *Opiate*. Meconium was obtained from 61 infants and analyzed for opiate by FPIA and RIA. The concordance of positive and negative results were 95% and 98%, respectively.

FPIA	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%)

Meconium was also obtained from 20 infants and analyzed for morphine by FPIA and GC/MS.

The results show high concordance between positive and negative tests by the 2 methods as shown in the following table.

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

F. Effect of Carryover.

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

G. 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, opiate or cannabinoid
4. Technical or procedural errors.

H. 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. Pairs of control tubes may be spaced throughout the assay to help verify the absence of significant drift. Inspect the results for agreement within tube pairs, and take care to avoid carry-over from sample to sample.

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. (J.0. Westgard et al, "Multi-rule chart for quality control" Clinical

Chemistry 27 (1981) 493-501. See also Scandinavian Journal of Clinical and Laboratory Investigation 44 (1984) Suppl 171 and 172. Repeat samples are a valuable additional tool for monitoring interassay precision.

4. Data Reduction: It is good practice to construct a graph of the calibration curve as a visual check on the appropriateness of the transformation used, even where the calculation of results is handled by computer. See further S.E. Davis et al, "Radioimmunoassay data processing with a small programmable calculator" Journal of Immunoassay 1(1980) 15-25; and R.A. Dudley et al, "Guidelines for immunoassay data reduction" Clinical Chemistry 31 (1985) 1264-71.

References

1. Ostrea EM, Parks P, Brady M. Rapid isolation and detection of drugs in meconium of infants of drug dependent mothers. Clin Chem 1988; 34: 2372-2373.

2. Ostrea EM, Brady MJ, Parks PM, Asensio DC, Naluz A. Drug screening of meconium in infants of drug dependent mothers. An alternative to urine testing. J Pediatr 1989; 115:474-477.

3. Ostrea EM, Lynn SN, Wayne RFL, Stryker JC. Tissue distribution of morphine in the newborns of addicted monkeys and humans. Dev Pharmacol Ther 1980;1:163-170

4. Lucena J, Silvestre MA, Raymundo AL, Roxas R, Ostrea EM. The effect of timing, dosage and duration of cocaine intake during pregnancy on the amount of cocaine in meconium in a rat model. Pediatr Res 1991;29:62A.

5. Silvestre MA, Lucena J, Ostrea EM. The effect of timing, dosage and duration or morphine intake during pregnancy on the amount of morphine in meconium in a rat model. Biol Neonate 1997;72: 112-117

6. Ostrea EM, Brady MJ, Gause S, Raymundo AL, Stevens M. Drug screening of newborns by meconium analysis: A large scale, prospective, epidemiologic study. Pediatrics 1992; 89:107-113.

7. Ostrea EM, Martier S, Welch R, Brady MJ. Sensitivity of meconium drug screen in detecting intrauterine drug exposure of infants. Pediatr Res 1990; 27:219A.

8. Callahan CK, Grant TK, Phipps P, Clark G et al. Measurement of gestational cocaine exposure: Sensitivity of newborn hair, meconium and urine. J Pediatr 1992;120:763-8.

9. Maynard EC, Amuroso LP, Oh W. Meconium for drug testing. Amer J Dis Child 1991;145:650-652.

10. Ostrea EM, Romero A. Adaptation of the meconium test for mass drug screening. J Pediatr 1993;122:152-154.

11. Ostrea EM, Romero A. Selection criteria for routine drug screening of infants by meconium analysis. Pediatr Res 1992;31:215A.

12. Ostrea EM, Lizardo E, Tanafranca M. The prevalence of illicit drug exposure in infants in the NICU as determined by meconium drug analysis. Pediatr Res 1992;31:215A.

13. Knapp DK, Ostrea EM, Romero A. Fetal exposure to nicotine in passive and active maternal smoking as detected by meconium analysis. J Pediatr 1994;124:471-6.

14. Bandstra ES, Steele BW, Chitwood DD et al. Detection of in utero cocaine exposure: A comparative methodologic study. Pediatr Res 1992;31:58A.

15. Ostrea EM, Knapp DK, Ostrea AR, Tannenbaum L, Salari V. A prospective study comparing systematic interview and analysis of maternal hair and meconium to determine illicit drug use during pregnancy. Pediatr Res 1994;35:245A

16. Montes M, Romero A, Ostrea EM, Ostrea AR. Improved method of GC/MS analysis of meconium for opiate, cocaine and cannabinoid. Pediatr Res 1993;33:66A.