Mectest Cannabinoid Radioimmunoassay (MTHCR)

Intended Use:
Double Antibody Cannabinoids is an ^{125}I radioimmunoassay designed for the semiquantitative measurement of cannabinoids in meconium. It is intended strictly for in vitro use in the context of a program involving an established confirmatory kit for tetahydrocannabinol (THC, cannabis, marijuana) and its metabolites.

Catalog number: MTHCR (l00 tubes)
The 100 tube kit contains less than 7 microcuries (259 kilobecquerels) of radioactive ^{125}I THC. The Double Antibody Cannabinoids assay provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Introduction:
Cannabis sativa is a weedy annual, which grows readily in both tropical and temperate areas of the world. Delta 9-tetrahydrocannabinol (THC) is the primary psychoactive ingredient found in the leaves and flowering tops of the plant, preparations of which are commonly referred to as marijuana.

Marijuana is usually either ingested orally or inhaled by smoking. THC, being very lipophilic, accumulates in body fats, a property that contributes to the very long urinary elimination times seen with this drug. Storage of the drug in body fat may be faster than elimination in chronic users, and this may lead to longer elimination times in these individuals compared to occasional users. When smoked, THC rapidly enters the bloodstream (1-3 minutes) whereas when taken orally, the absorption is considerably slower (1.5-3 hours). Once in the bloodstream, it is quickly transformed by liver enzymes to several metabolites, the foremost is 11-nor-delta 9-THC-9-carboxylic acid. In man, two-thirds of the dose is excreted in the feces and a third in the urine, almost entirely as metabolite. THC itself is detectable for a few hours in blood, but because of its rapid metabolism and distribution, little unchanged THC appears in the urine.

Marijuana, when smoked by the pregnant woman, crosses the placenta readily due to its lipophilic nature. Fetal metabolism of marijuana is through the liver and the metabolites are secreted into the bile or urine. The presence of cannabinoid metabolites in fetal meconium is secondary to their deposition in the fetal intestines either from bile secretion or fetal swallowing of amniotic fluid which contains fetal urine.

DPC's Double Antibody Cannabinoids procedure is a competitive radioimmunoassay in which ^{125}I THC competes with cannabinoids in the patient sample for antibody sites. After incubation for a fixed time, separation of bound from free is achieved by the phosphoethylene glycol (PEG) accelerated double antibody method. Finally, the antibody-bound fraction is precipitated and counted. Patient sample concentrations are read from a calibration curve.

Procedure:
The assay requires a total incubation time of only 75 minutes. An experienced technician with proper equipment can prepare a calibration curve and 20 patient samples for counting in 2 hours, using less than 1 hour of bench time. Sample and tracer additions can be handled simultaneously, if desired, with the help of an automatic pipettor-diluter.

Separation:
Separation is by a single-reagent Precipitating Solution consisting of second antibody and dilute PEG. This method has been shown to yield more consistent and reproducible results than other liquid-phase techniques. The flocculation reaction is complete in just minutes at room temperature. The precipitate packs to a firm and easily visible pellet, and nonspecific binding is at least as low as in separations employing second antibody alone.

Data Reduction:
Conventional RIA techniques of calculation and quality control are applicable. The assay has been optimized for linearity in a logit-log representation throughout the range of its calibrators.

Calibration:
The kit is equipped with calibrators having THC metabolite values ranging from 10 to 250 ng/mL of 11-nor-delta 9-THC-9-carboxylic acid. The calibrators are lyophilized for maximum stability.

Counts:
The tracer has a high specific activity, with total counts of approximately 90,000 cpm at iodination. Maximum binding is approximately 50-60%. Nonspecific binding and patient blanks are negligible.

Precision:
CVs are low and uniform.

Specificity:
The antiserum is highly specific for cannabinoids, with very low cross-reactivity to other compounds that might be present in patient samples.

Accuracy:
The assay calibrators have been checked against the National Bureau of Standards Standard Reference Material for THC Metabolite in Urine. Recovery experiments have shown that the assay is accurate over a broad range of THC metabolite values. Its accuracy has been further verified in patient comparison studies against GC/MS.

Warnings and Precautions:
For in vitro diagnostic use. Before opening the kit, review the paragraphs on safety printed on the inside front cover, as they relate to the safe handling and disposal of reagents containing radioactivity, human serum and sodium azide. Prepare all components at least 10 minutes prior to use.

Materials Supplied:

Initial Preparation

1. THC Antiserum (MTHCR1):
One vial of THC antiserum raised in goats and supplied in liquid form, ready to use. Each vial contains 20 mL. Store refrigerated: stable at 2-8 °C for at least 30 days after opening. Before use, antiserum should be thoroughly mixed (without foaming) since a fine precipitate may form after storage at 2-8 °C.

2. ^{125}I THC (MTHCR2):
One vial of iodinated tetrahydrocannabinoic acid supplied in liquid form, ready to use. Each vial contains 20 mL. Store refrigerated: stable at 2-8 °C for at least 30 days after opening, or until the expiration date marked on the vial. Before use, tracer should be thoroughly mixed (without foaming) since a fine precipitate may form after storage at 2-8 °C.

3 THC Calibrators (MTHCR3):
One set of six vials labeled A through F, of lyophilized 11-nor-delta 9-THC-9-carboxylic acid calibrators. At least 30 minutes before use, reconstitute the zero calibrator **A with 2.0 mL** distilled water, and the remaining calibrators **B through F with 1.0 mL** distilled water. Use volumetric pipets and mix by gentle swirling. Store refrigerated: stable at 2-8 °C for at least 30 days after reconstitution. The life of the calibrators may be extended by freezing. Aliquot if necessary to avoid repeated thawing and freezing.

Because delta-9-tetrahydrocannabinoic acid may be lost to glass and plastic surfaces at low concentrations, transfer of calibrator solutions should be minimized and silanized glass vials and pipets should be used. Vigorous mixing and freeze-thaw cycles should be avoided. Delta-9-tetrahydrocannabinoic acid solutions may not show dilutional parallelism, but it can be demonstrated with delta-8-tetrahydrocannabinoic acid solutions such as ConDOA-T (TOXC).

The calibrators contain respectively 0, 10, 25, 50, 100 and 250 ng/mL of 11-nor-delta 9-THC-9-carboxylic acid with preservatives (nonazide bacteriostatic agents). Intermediate calibration points may be obtained by mixing calibrators in suitable proportions.

4. Precipitating Solution (MTHCR4):
One vial of Precipitating Solution consisting of donkey anti-sheep gamma globulin (DASGG) and dilute polyethylene glycol in saline. Each vial contains 110 mL. The Precipitating Solution is applied in liquid form, ready to use. Store refrigerated: stable at 2-8 °C for at least 30 days after opening. Since a fine precipitate may form after refrigeration, the precipitating solution should be thoroughly mixed before use, without foaming.

Materials Required But Not Provided:

1. Cannabinoid meconium controls:
Control 1. Emulsify 0.5 g of drug free meconium in 5 mL of meconium solvent in the meconium processor (see procedure below for meconium collection and processing). Add 300 microliters of THC calibrator (250 ng/mL): final THC concentration is 14.1 ng/mL.
Control 2 Emulsify another 0.5 g of control (drug free) meconium in 5 mL of meconium solvent and add 1 mL of THC calibrator (250 ng/mL): final THC concentration is 41.7 ng/mL. Process meconium controls 1 and 2 as below (see Meconium processing).

2. Gamma counter -compatible with standard 12x75 mm tubes.

3. Centrifuge - preferably refrigerated and capable of at least 1500 g.

4. Vortex-mixer.

Note: Additional calibrators can be purchased through Mectest Corporation.

Reagent Preparation:

1. Distilled or deionized water.

2. Volumetric pipets: 1 or 2 mL.

Radioimmunoassay:

1. Plain 12x75 mm polypropylene tubes - for use as NSB tubes.

2. Micropipets: 50 µL, 100 µL, 200 µL and 1000 µL. For the 200 µL reagent additions, a reliable repeating dispenser (Nichiryo or equivalent) is recommended. A syringe style dispenser (Nichiryo, Cornwall or equivalent) accurate to within ± 0.05 mL is recommended for the 1.0 mL addition of Precipitating Solution.

3. Foam decanting rack - available from Diagnostic Products Corporation, 5700 West 96th St., Los Angeles, CA 90045.

Procedure for the Preparation and Analysis of Cannabinoid in Meconium

A. <u>Meconium Collection</u>:

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 (see below).

2. Unscrew cap of MECTEST meconium processor. Set tube in a rack (Do not spill reagent).

3. Use scoop attached to the cap to obtain 0.5 g of meconium (approximately one large, scoopful of meconium).

4. Place cap and scoop containing meconium back into the MECTEST processor.

5. 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. <u>Meconium Processing:</u>

1. Vortex MECTEST processor with scoop acting as a stirrer, until meconium is well 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. to prevent overheating. Repeat centrifugation at 4600 rpm for another 30 min.

6. Collect total ultrafiltrate (approximately 250 microliters) for drug analysis.

Note: Meconium ultrafiltrate with cannabinoid concentrations greater than that of the highest calibrator in the assay should be diluted with the kit's zero calibrator to bring the sample within the range of the calibrators.

C. Radioimmunoassay:

1. Set up and label as many tubes as are required for duplicate Total, NSB, Calibration Standards (A-F), meconium ultrafiltrates (unknown specimens) and meconium controls numbered "1" and "2" to be assayed.

Calibrator	THC Metabolite
A(MB)	0
B	10
C	25
D	50
E	100
F	250

2. Add 50 microliters each of the controls and the unknown to the appropriate test tubes. Pipet 50 microliters of the A Calibrator into the NSB and A tubes. Pipet 50 microliters of the B through F Calibrators into their appropriate tubes. Run the total, calibrators, unknown and controls at least in duplicate. (Samples found to contain high concentrations. outside of the range of the Calibrators can be diluted in the zero calibrator before assay).

3. Dispense 200 microliters of ^{125}I THC to all tubes. Pipet directly to the bottom of the tubes. Vortex.

4. Dispense 200 microliters of THC Antiserum to all tubes except the Total and NSB tubes. Vortex.

5. Incubate tubes at room temperature for 1 hour.

6. Add 1 mL of Precipitating Solution to all tubes except the Total tubes. Vortex.

7. Incubate for 15 minutes.

8. Centrifuge tubes for 15 minutes at 3000xg preferably using a refrigerated centrifuge.

9. Set aside the Total tubes and decant the rest of the tubes. Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tubes) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

10. Count for 1 minute in a gamma counter.

D. Quantitative Analysis:

1. To calculate THC concentrations from a logit-log representation of the calibration curve, first calculate for each pair of tubes the average NSB-corrected counts per minute:

Net Counts = Average CPM - Average NSB CPM

Then determine the binding of each pair of tubes as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net MB Counts}} \times 100$$

The calculation can be simplified by omitting the correction for nonspecific binding (NSB): samples within range of the calibrators yield virtually the same results when Percent Bound is calculated directly from Average CPM. Using the logit-log graph paper provided with the kit, plot Percent Bound on the vertical axis against Concentration on the horizontal axis for each of the calibrators B through F, and draw a straight line approximating the path of these five points. Cannabinoid concentrations for the unknowns may then be estimated from the line by interpolation. Although other approaches are acceptable, data reduction by the logit-log method just described has certain advantages in this context for example, in allowing easier recognition of deviant calibration points since the procedure has been optimized for linearity in that representation.

Example: The values below are intended for illustration only and not to calculate results for an assay.

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	THC Metabolite (ng/mL)
T	87,767				
	87,732	87,750			
NSB	2120				
	1981	2051	0		
A(MB)	49,687				
	49,523	49,605	47,554	100%	0
B	37,354				
	37,159	37,257	35,224	74.1%	10
C	28,352				
	28,336	28,334	26,293	55.3%	25
D	21,528				
	21,228	21,528	19,477	41.0%	50
E	15,805				
	15,655	15,730	13,679	28.8%	100
F	9,659				
	9,636	9,648	7,597	16.0%	250
Unknowns					
X1	30,894				
	30,626	30,760	28,709	60.4%	20
X2	26,866				
	26,833	26,850	24,799	52.1%	30
X3	12,809				
	12.760	12,785	10,734	22.6%	144

Quality Control Parameters:

T=87,750 cpm.
%NSB=2.3%
%MB=54%
20% Intercept=173 ng/mL
50% Intercept=33 ng/mL
80% Intercept=6 ng/mL

E. Reporting of Results:

Negative test: cannabinoid concentrations in meconium that are below the cut off concentration are reported as:

Negative for cannabinoid
Cut off concentrations
(minimum drug detectable) = 6.54 ng/mL

Warning:
A negative result does not eliminate the possibility of consumption of illicit drugs by the mother. The formation of meconium starts at the 12th week of gestation. Thus, illicit drug use by the mother during the first trimester of pregnancy may not result in a positive meconium drug test. Similarly, meconium samples may not all be positive for drugs if the mother has only been an episodic user of drugs. Thus, pooling of meconium obtained from a number of diapers will increase the likelihood of a positive test. The results of the meconium drug test should also be correlated to the maternal history and to toxicological tests that have been done on the mother. When meconium is used in any other systems/methods for the detection of drugs of abuse, the user must be aware that the performance characteristics of such systems/methods have not been determined by the manufacturer nor have been FDA cleared. Likewise, the concentration of the drug of abuse in meconium using such systems/methods have not been correlated with the dose of the drug of abuse consumed by the mother nor with the clinical picture in the neonate.

Positive test:
Cannabinoid concentrations at or greater than the cut off concentrations are reported as:

Presumptive positive for cannabinoid
Cut off concentrations
(minimum drug detectable) = 6.54 ng/mL

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

I. Sensitivity
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 the radioimmunoassay. 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 radioimmunoassay 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-tetrahydrocannabinol-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).

II. Specificity
Cross reactivity:
Meconium was emulsified using the MECTEST Processor and tested for the presence of endogenous compounds. Meconium was also spiked with cocaine, morphine and cannabinoid at concentrations ranging from 0 to 423 ng/mL, as well as the following drugs, which were prepared at concentrations of 100,000 ng/mL, namely: acetaminophen, phenobarbital, acetylsalicylic acid, propoxyphene, pentazocine, chlorpromazine, ibuprofen, meperidine, diazepam, lidocaine and caffeine.

Meconium contained endogenous amounts of bilirubin (60.23 ± 2.76 micrograms/gm meconium), blood (0 to +++ hemoglobin by qualitative guiac test) and protein (32.89 ± 12.36 micrograms/gm meconium) which did not interfere in the recovery of cannabinoid in meconium. Similarly, cross-reaction between cocaine, opiate or cannabinoid at the concentrations used was not observed. Recovery rate was 96.6 ± 6.2% for cannabinoid. There was 0% to 0.2% cross-reactivity of cannabinoid to the following drugs which were prepared at concentrations of 100,000 ng/mL: acetaminophen, phenobarbital, acetylsalicylic acid, propoxyphene, pentazocine, chlorpromazene, ibuprofen, meperidine, diazepam, lidocaine and caffeine.

Cross reactivity between various cannabinoid metabolites			
Compound	ng/mL added	Apparent Conc (ng/mL)	Percent Crossreactivity
11-nor-delta 9-tetrahydro-cannabinol-9-carboxylic acid	100	100	100%
Cannabinol	10,000	3.4	0.03%
	100,000	24	0.02
Cannabidiol	10,000	5.5	0.06%
	100,000	21	0.02%
Delta-8-tetrahydrocannabinol	100	>250	>100%
	1,000	>250	>100%
Delta-8-tetrahydrocannabinol -11-oic acid	10	9.7	97%
	100	125	125%
	1,000	>250	>100%

III. Precision
Interassay precision:
Known amounts of 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 processed individually using the MECTEST processor and analyzed for cocaine, morphine and cannabinoid by radioimmunoassay. The interassay coefficient of variability (CV) of cannabinoid was 8.91%.

Intra-assay precision:
To determine 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 5.3 ± 2.6 % for cannabinoid.

To compare "within sample" drug concentration, meconium from 2 infants were sampled at two sites per specimen and tested for cannabinoid by radioimmunoassay. The results (see below) showed varying concentration of drugs per site within samples. This indicates that drugs are unevenly distributed in meconium. Thus, for appropriate sampling, meconium has to be mixed well before an aliquot is taken.

Comparison of cannabinoid concentrations in meconium at 2 sampling sites (Sites A and B)

Cannabinoid concentration (ng/mL)		
	Site A	Site B
Specimen 1	46.00	40.30
Specimen 2	149.36	76.86

IV. Stability of Cannabinoid In Meconium

The stability of cannabinoid in meconium was 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 5 months.

1. Meconium allowed to stand at room temperature for 24 hours resulted in a 30% decrease in cannabinoid concentration. Thus, meconium should be sampled and processed as soon as possible after its excretion to avoid loss of drugs.

Effect on cannabinoid concentration if meconium is stored at room temperature for 24 hours

Cannabinoid concentration (ng/mL)			
	0 hour	24 hours	% change
Sample 1	46.00	98.80	+114.5
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 showed relative stability in cannabinoid concentration. This indicates that the transport of meconium samples is best undertaken by prior emulsification of meconium in MECTEST solvent.

Effect on cannabinoid concentration if meconium is emulsified in MECTEST solvent at room temperature for 72 hours

Cannabinoid concentration (ng/mL)			
	0 hour	72 hours	% change
Sample 1	46.00	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. The effect of freezing on cannabinoid concentration in meconium is shown in the table. At -15^{o} C, cannabinoids are stable in meconium for at least 9 months.

Effect of freezing at -15'C on cannabinoid concentration in meconium			
Cannabinoid concentration (ng/mL)			
Sample	Interval between	Cannabinoid (ng/mL)	
Number	tests (days)	Original conc	Conc after freezing
1	322	0	0
2	322	0	0
3	322	0	0
4	170	0	0
5	170	77	42
6	170	101	96
7	170	297	278
8	109	0	0
9	120	0	0
10	109	0	0
11	109	0	0
12	109	0	0
13	109	0	0

V. Clinical Study:

Fifty meconium samples from in utero drug exposed and control infants were analyzed for cannabinoid by radioimmunoassay and gas chromatography/mass spectrometry (GC/MS) [16]. The table shows agreement between the RIA and GC/MS results.

Analysis of 50 meconium samples for cannabinoid by RIA and GC/MS					
Sample	RIA (ng/mL)	GC/ MS	Sample	RIA (ng/mL)	GC/MS
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	17.0	+
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	-

VI. Effect of Carryover:

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

VII. Limitations:

Based on a review of the literature, the following may cause false positive reactions:

1. Passive maternal inhalation of cannabinoid.

2. Technical or procedural errors.

VIII. 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 kits 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 carryover 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 carryover 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.

Q.C. Parameters: We recommend keeping track of the following performance measures.

* T = Total Counts (as counts per minute)

$$\%NSB = 100 \times \frac{\text{Average NSB Counts}}{\text{Total Counts}}$$

$$* \ \%MB = 100 \times \frac{\text{Average MB Counts - Average NSB Counts}}{\text{Total Counts}}$$

And the 20, 50 and 80 percent "intercepts," where *20% = THC Metabolite Concentration at 20 Percent Bound, etc.

5. Centrifugation:
The procedure calls for centrifuging at 4600 rpm x 30 minutes. Lower accelerations are satisfactory only if the centrifugation time is increased appropriately. A high-speed refrigerated centrifuge is desirable but not essential.

IX. 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[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 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 methodological 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. [14]

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.

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