

Field Testing the Microgenics DRI® EtG assay for Georgia’s Treatment Courts: A 3-Month Analysis of Screening and Confirmation Data

DeKalb County Drug Court, 556 N. McDonough Street, Decatur, Georgia 30030. The report was prepared for the Georgia Administrative Office of the Courts and Judicial Council Standing Committee of Drug Courts. *The study was conducted with the generous support of United States Drug Testing Laboratories, Inc.*

EXECUTIVE SUMMARY

Ethyl Glucuronide (EtG) is a direct biomarker of ethanol ingestion with a 2 to 5 day detection window in urine and is generally accepted to detect beverage ethanol consumption at or above a 90 percent sensitivity level. EtG’s superior detection window and sensitivity over conventional breath and urine ethanol detection methods has made it an increasingly popular assay for detection of participant alcohol use and relapse among Georgia’s Drug and DUI treatment courts. The introduction of the Microgenics DRI® EtG assay was met with considerable interest by Georgia’s Drug and DUI Treatment Courts given its potential cost-savings and operational benefits. However, the limited public domain data exploring the screen’s field performance and relationship to mass spectrometry confirmation results prompted the DeKalb County Drug Court to undertake the present study with the participation of several partnering jurisdictions, including Cherokee, DeKalb, Fulton, and Gwinnett County treatment courts.

The primary goal of the study was to confirm the Microgenics DRI® EtG assay’s *sensitivity, specificity, and reliability* in non-laboratory conditions. The study was conducted by comparing positive results at 500 ng/mL and 100 ng/mL to higher-order mass spectrometry confirmation results on the same sample. Although a relatively limited number of specimens tested positive at the 500 ng/mL cut-off, the Microgenics DRI® EtG assay stood-up to LC/MS/MS in approximately 85% of confirmations. Conversely, the screen did not perform as well at a 100 ng/mL cut-off, yielding a 59% confirmation rate. A rather startling result from the study was that nearly 12% of screens sent for LC/MS/MS confirmation indicated the possibility of EtG breakdown between screen and confirmation.

The results of the study suggest that the Microgenics DRI® EtG assay performs at a satisfactory level of sensitivity, specificity and reliability at 500 ng/mL in non-laboratory conditions.

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INTRODUCTION

Ethyl Glucuronide (EtG) is a direct biomarker of ethanol ingestion with a 2 to 5 day detection window in urine and is generally accepted to detect beverage ethanol consumption at or above a 90 percent sensitivity level¹. EtG’s superior detection window and sensitivity over conventional breath and urine ethanol detection methods has made it an increasingly popular assay for detection of participant alcohol use and relapse among Georgia’s Drug and DUI treatment courts.

The chief concerns for Georgia’s treatment courts regarding reliance on EtG, as an alcohol detection methodology, are cost and sensitivity to unintentional ethanol exposure or non-beverage alcohol. EtG is tested using an expensive liquid chromatography-tandem mass spectrometry (LC/MS/MS), which typically costs \$20 to \$40 per specimen, and requires samples to be sent “chain of custody” to a reference lab with a 2 to 5 day waiting period for results. The issue of EtG sensitivity to non-beverage alcohol, such as commercial and personal care items, prompted the Center for Substance Abuse Treatment (CSAT) to release a *Substance Abuse Treatment Advisory* (2006), cautioning against legal or disciplinary action based solely on EtG, until further study is undertaken and reliable cut-offs can be set to guard against non-beverage exposure to alcohol². Concerns about EtG’s sensitivity to non-beverage consumption is further compounded by evidence of in-vitro production of EtG related to the presence of yeast infection and documented post-collection synthesis or breakdown of EtG (bacterial glucuronidase degradation) in *E. coli*-infected samples³.

¹ Wurst F; Seidl S; Ladewig D; Muller-Spahn F; Alt A. Ethyl Glucuronide: on the Time Course of Excretion in Urine During Detoxification. *Addiction Biology* 2002, 7, 427-434

² Center for Substance Abuse Treatment. The Role of Biomarkers in the Treatment of Alcohol Use Disorders. *Substance Abuse Treatment Advisory*. DHHS Publication No. 06-4223. Volume 5, Issue 4, September 2006

³ Helander, Anders; Olsson, Ingrid; Dahl, Helen. Postcollection Synthesis of Ethyl Glucuronide by Bacteria in Urine May Cause False Identification of Alcohol Consumption. *Clinical Chemistry* 2007, 53 (10), 1855-1857

Remarkably, neither the high cost of mass spectrometry, proven sensitivity to non-beverage alcohol, nor the risk of false-positives or negatives owing to medical issues has curbed the widespread use of the EtG biomarker in Georgia's treatment courts or elsewhere in the country. Many of Georgia's treatment courts have taken the position that EtG's ability to detect alcohol use has been field-proven, despite the paucity of supporting empirical data and the prevailing concerns about cut-offs. In fact, most treatment courts maintain a zero tolerance policy and take the position that participants are responsible for avoiding any contact with alcohol, as they are with controlled substances. The position is defensible to the extent that no study has demonstrated that non-beverage exposure to alcohol yields EtG concentrations above the relatively conservative 500 ng/mL cut-off adopted by most courts. Granted, some Georgia courts do maintain a sub-500 ng/mL EtG cut-off, and the risk of non-beverage positives is a factor, although there are no successful challenges on record.

In early 2007, ThermoFisher Scientific, a nationally-known biomedical company, released the Microgenics DRI® EtG assay (*FDA approval pending*), which is designed to screen for EtG in urine using a homogeneous enzyme immunoassay lab technology at a 500 ng/mL cut-off. The immunoassay is a relatively fast, inexpensive, and reliable way of testing for conventional drugs of abuse, although the more *sensitive* and *specific* mass spectrometry confirmation is generally required by most jurisdictions for contested screens. The introduction of the Microgenics DRI® EtG assay was met with considerable interest by Georgia's Drug and DUI Treatment Courts given its potential cost-savings and operational benefits. However, the limited public domain data exploring the screen's field performance and relationship to mass spectrometry confirmation results prompted the DeKalb County Drug Court to undertake the present study with the participation of several partnering jurisdictions, including Cherokee, DeKalb, Fulton, and Gwinnett County treatment courts.

The primary goal of the study was to confirm the Microgenics DRI® EtG assay's sensitivity, specificity, and reliability by comparing positive results at 500 ng/mL to higher-order mass spectrometry confirmation results on the same sample. Given that a number of treatment courts utilize a lower 100 ng/mL cut-off, specimens indicating the presence of EtG concentrations between 100 and 500 ng/mL were randomly selected and sent for mass spectrometry confirmation to explore the screen's reliability sub-500 ng/mL. Beyond relatively tight collection and delivery protocols, the study relied on specimens collected under normal field conditions with the hope of yielding "real world" performance. Additionally, with the recently documented risk of EtG breakdown or post-collection synthesis, specimens were also tested for the presence of a

second ethanol biomarker, Ethyl Sulfate (EtS), which yields a comparable detection window and sensitivity to EtG⁴.

METHODOLOGY

EtG was measured in urine samples collected in multiple Georgia treatment court collection sites over a 3-month period in 2007. Upon collection of the samples by the participating treatment courts, the samples were kept at room temperature until they were prepared for delivery and transported to the DeKalb County Drug Court (DCDC) Laboratory by either personal courier or through the postal mail service. Once the samples were received by DCDC, they were stored at room temperature and tested using the Microgenics DRI® EtG Enzyme Immunoassay on an Olympus AU400 platform with standard protocols. Assay results were semi-quantitative (0, 100 [LLOQ], 500, 1000, 2000 [ULOQ] ng/mL) with 4 QC levels (100, 375, 625, 750 ng/mL). Positive EtG results at or above 500 ng/mL and test results indicating concentrations between 100 and 500 ng/mL were sent to United States Drug Testing Laboratories, Inc (USDTL) for LC/MS/MS. EtG and EtS determinations were performed at USDTL using standard protocols on an API 2000 LC/MS/MS.

The Georgia treatment court collection sites included the Cherokee County DUI Court, DeKalb County Drug Court, DeKalb County DUI Court, Fulton County Drug Court, and Gwinnett County DUI Court. Participants were required to provide a specimen under direct observation, divide it into two collection cups, and seal it with tamper evident seals. The two sealed cups were then placed in a specimen bag along with an absorbent pad. As a control measure, the participating courts used the same lab kit materials provided by Substance Abuse Specialties, Inc. A chain of custody form was placed in the front envelope area of the specimen bag. The chain of custody forms were completed by the participant to document all prescribed and other medicines the participant may have taken. The chain of custody form consisted of an original and three attached carbonless copies. The original and one copy were delivered to the DCDC laboratory, along with the split cup, A and B sample. The second copy was maintained by the participating court and the third and final copy was given to the participant.

Once the specimens arrived at the DCDC laboratory, the chain of custody forms were removed from the specimen bags and data were input into a database. The samples were removed from the bags and separated into A and B samples. The A samples were unsealed and aliquoted

⁴ Helander, Anders and Dahl, Helen. Urinary Tract Infection: A Risk Factor for False-Negative Urinary Ethyl Glucuronide but Not Ethyl Sulfate in Detection of Recent Alcohol Consumption. *Clinical Chemistry* 2005, 51 (9), 1728-1730

for testing, while B samples were refrigerated at approximately 6 degrees Celsius. When a specimen tested positive at 500 ng/mL, the DCDC laboratory automatically performed a repeat analysis. All specimens that initially tested positive also tested positive during the repeat analysis. Once the repeat analysis was confirmed, the B sample was forwarded to USDTL for LC/MS/MS. Additionally, samples indicating concentrations below 500 ng/mL or “error” results were randomly selected for LC/MS/MS.

RESULTS

The DeKalb County Drug Court Laboratory collected and ran 1576 screens from the five participating Georgia treatment courts. Transport time from collection to screen, and screen to confirmation were recorded. EtG screen results and its corresponding EtG and EtS confirmation results were also recorded. For calculating purposes error messages that were sent for confirmation were not included in our findings. Two Receiver-Operating Characteristic (ROC) curves were graphed using 98 samples. An additional two ROC curves using 91 samples were graphed backing out seven cases due to the possibility of EtG breakdown. A Bayesian Analysis was conducted to provide an estimate of screen reliability (positive-predictive and negative-predictive values), sensitivity (true-positives), and specificity (true-negatives).

Overall, 119 specimens were sent to USDTL for LC/MS/MS. The average transport and shelf-time for specimens was two days from collection to screen at the DCDC Laboratory and six days from screen to confirmation.

Table 1 – Number of days in transport and shelf-time

	Range	Mean	Median	Mode
Collection to Screen	0-15	2.36	2	1
Screen to Confirmation	1-19	6.48	6	6

Sixteen specimens screened positive at or above 500 ng/mL with the Microgenics DRI® EtG assay, and 11 (69%) were confirmed by USDTL at or above 500 ng/mL (See Appendix 1). An additional specimen yielded a LC/MS/MS result above 200 ng/mL, but was not considered positive to preserve a consistent cut-off between the screening and confirmation technologies. Three of the non-confirmed screens were positive for EtS (25 ng/mL cut-off), suggesting the possibility of EtG breakdown between screen and confirmation. It should be noted that the turn-around from screening to confirmation reveals that the three specimens exceeded the study’s mean time-frame by one to two days, but the present study does not provide a basis to assess the impact of the delay. Controlling for the three cases of possible EtG breakdown, the Microgenics DRI® EtG assay’s reliability with LC/MS/MS was estimated at 85%.

The study found 70 screens with EtG concentrations above 100 ng/mL with the Microgenics DRI® EtG assay and 54 between 100 and 500 ng/mL were randomly selected and sent for confirmation during the 3-month collection period (See Appendix 2). Thirty-seven (53%) were confirmed by USDTL using LC/MS/MS at or above 100 ng/mL. Seven of the non-confirmed screens tested positive for EtS, suggesting the possibility of EtG breakdown. The confirmed EtG concentrations between 100 and 500 ng/mL combined with the four EtG negative/EtS positive confirmations provide a modest increase to the confirmation rate, bringing the Microgenics DRI® EtG assay's sub-500 ng/mL cut-off reliability with LC/MS/MS to 59%.

Twenty-eight specimens were randomly drawn from a pool of 1456 with EtG concentration below 100 ng/mL and sent for LC/MS/MS. Twenty-seven (96%) were confirmed negative (See Appendix 3).

It is worth noting that 23 (1%) of the 1576 screens resulted in errors at the DCDC Laboratory and were, thereby, suspect. Twenty-one specimens were forwarded to USDTL for LC/MS/MS. Only one came back with an EtG concentration above 500 ng/mL. However, four samples confirmed as EtG negative with an EtS positive, suggesting breakdown occurred (See Appendix 4).

The ROC curves found on Appendices 5 and 6 define the sensitivity and specificity indicators for each confirmation. The ROC curves represent the ratio of true positives to false positives present in a given sample set. The highest level of accuracy for the ROC curve is found at the closest plot to the upper left corner. In both Appendix 5 and Appendix 6 the top line plots the confirmation results using the 500 ng/mL screen cut-off. The bottom line plots the confirmation results using the 100 ng/mL screen cut-off. Both line charts also reveal where the 500 ng/mL and 100 ng/mL cut-off actually occurs. Appendix 5 uses the 98 screen to confirmation sample set and Appendix 6 uses the 91 screen to confirmation sample set. As seen on both ROC charts, sensitivity and specificity are closer at the 500 ng/mL cut-off than at the 100 ng/mL cut-off indicating a higher level of accuracy, which in turn denotes a more defensible cut-off.

Another method often used to evaluate sensitivity, specificity, and overall reliability is a Bayesian Analysis. Whereas, ROC curves graph each result from a particular sample set, a Bayesian Analysis statistically evaluates the sample set as a whole. By using a Bayesian Analysis we calculated sensitivity, specificity, positive predictive value, and negative predictive value at the 500 ng/mL and 100 ng/mL cut-offs. While the ROC curves yield a picture of screen sensitivity and specificity, the Bayesian Analysis provides an estimate of reliability. Reliability is determined through the positive and negative predictive values. The positive predictive value

indicates at what level of probability the screening method of true positives will confirm. Whereas, the negative predictive value indicates at what level of probability the screening method of true negatives will confirm.

The Bayesian Analysis indicates that the screen's positive predictive value lies between 69% and 85% for the 500 ng/mL cut-off, while the positive predictive value lies between 53% and 59% for the 100 ng/mL cut-off. (See Appendix 7 and 8). In both cases, the higher estimate controls for possible EtG breakdown and represents the presence of EtS. The negative predictive value for both the 500 ng/mL cut-off and the 100 ng/mL cut-off was over 95%. As with sensitivity and specificity the higher level of reliability is indicated at the 500 ng/mL cut-off.

DISCUSSION

Although a relatively limited number of specimens tested positive at the 500 ng/mL cut-off, the Microgenics DRI® EtG assay stood-up to LC/MS/MS in 69% of confirmations. Controlling for the possibility of EtG breakdown, as evidenced by EtG negative/EtS positive results, the confirmation rate rose to 85%, which is impressive performance under “real world” conditions with limited controls. The estimate would actually be 92% had one result not been set aside for confirming with a sub-500 ng/mL concentration.

The Microgenics DRI® EtG assay was examined by conducting two separate analyses, the ROC curve and a Bayesian Analysis. The methods revealed that sensitivity, specificity and reliability are most accurate at the 500 ng/mL cut-off. The main reason for confirming is to protect program integrity by eliminating false-positives; and as with other screening methodologies, the study's findings indicate that *sensitivity*, *specificity* and *reliability* are compromised by pushing for lower cut-offs.

Practitioners should take note that the assay is not marketed to be used with a sub-500 ng/mL cut-off, and its performance is compromised at the lower cut-off (53 to 59%). The risk of using a sub-500 ng/mL cut-off is that as false-negatives increase, the promised deterrent of the screen is likely to be decreased by repeatedly confronting participants with false-positives.

A rather startling result from the study was that nearly 12% of the 119 screens sent to USDTL for LC/MS/MS returned negative for EtG (100 ng/mL cut-off), but positive for EtS (25 ng/mL cut-off), indicating the possibility of EtG breakdown over a six day period between screen and confirmation. The rate of degradation is well above what was expected from the literature. It points to a serious concern for Georgia's treatment courts and speaks to the value of using reference labs that employ a dual-confirmation protocol, including EtG and EtS. Additionally, the finding supports the need to refrigerate samples, which has been found to retard EtG breakdown, and expedite transport to confirmation.

At roughly a third of the price of LC/MS/MS, the Microgenics DRI® EtG assay would seem to represent a way of increasing screening coverage and, thereby, detection and deterrent power for treatment courts that maintain a 500 ng/mL cut-off. The Microgenics DRI® EtG assay and the generation of lower-cost, semi-quantitative EtG screens that are certain to follow it to market, have great benefit for Georgia's treatment courts. As with all immunoassay screens, contested results should be confirmed by a more specific method such as LC/MS/MS, but it is safe to say that EtG, as a specific and sensitive indicator of alcohol consumption is a step forward. Additionally, EtG offers more advantages, such as a longer window of detection, over the conventional methods of breath, blood, and urine/ethanol. The study suggests that the Microgenics DRI® EtG assay performs at a satisfactory level of sensitivity, specificity and reliability in non-laboratory conditions. Georgia's courts can be assured that the screen represents a cost-effective, and empirically supported tool at 500 ng/mL to detect alcohol use and enhance program integrity.

SPECIAL ACKNOWLEDGEMENT

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APPENDICES:

Appendix 1:

Screen Results Above 500 ng/mL Sent for Confirmation

EtG Screen	EtG Confirmation	EtS Confirmation
514	847	1220
553	289	24.8
610	<LOD	16.8 fmr
770	1050	1040
834	1050	470
1053	1250	534
1417	1630	1270
1595	29 fmr	95
2287	2320	832
2607	96	1040
2861	9670	2360
2916	Detected at < 36	1700
3980	597	2870
4061	> 10000	9260
4567	>10000	4010
4987	>10000	>10000

LOD – level of detection

fmr – failed mass ratio

Appendix 2:

Screen Results Between 100-500 ng/mL Sent for Confirmation

EtG Screen	EtG Confirmation	EtS Confirmation	EtG Screen	EtG Confirmation	EtS Confirmation
100	102	32	101	<LOD	33 fmr
102	148	91	103	5 fmr	10 fmr
108	189	33	105	63.3 fmr	11.4 fmr
109	<LOD	1160	107	<LOD	<LOD
110	260	121	112	<LOD	31 qni
117	156	66	112	<LOD	7 fmr
117	127	144	114	8 fmr	18 fmr
141	99	106	123	<LOD	<LOD
154	156	93	126	<LOD	<LOD
156	274	279	131	84.9 fmr	20 fmr
159	17 fmr	107	132	30.9 fmr	17.5
163	158	169	155	<LOD	<LOD
188	221	152	165	24.7 fmr	3.38 fmr
191	<LOD	40	175	<LOD	42 fmr
192	198	56	185	56 fmr	20 fmr
194	401	131	197	<LOD	16 fmr
211	259	251	211	<LOD	<LOD
223	293	61	218	10.2 fmr	19.7 fmr
235	318	44	222	<LOD	<LOD
252	245	167	240	<LOD	24 fmr
252	358	324	313	44 fmr	9 qni
294	312	202	345	<LOD	<LOD
319	1500	57	414	29 fmr	75 fmr
325	536	584	449	<LOD	13 qni
337	619	506	466	34.2 fmr	51 fmr
349	426	278			
365	304	231			
481	464	199			
491	735	245			

LOD – level of detection

fmr – failed mass ratio

qni – qualifier not integrated

Appendix 3:

Screen Results Below 100 ng/mL Sent for Confirmation

EtG Screen	EtG Confirmation	EtS Confirmation
83	47 fmr	21
66	<LOD	<LOD
55	<LOD	<LOD
54	<LOD	<LOD
53	2.69 fmr	<LOD
51	9.93 fmr	<LOD
50	14 fmr	19 fmr
47	<LOD	<LOD
45	84	35
41	8 fmr	39 fmr
31	89	53
29	11.3 fmr	34.9 fmr
25	<LOD	<LOD
23	<LOD	17 fmr
23	6.05 fmr	26.6 fmr
17	<LOD	13 fmr
5	72 fmr	24 fmr
3	23.2 fmr	94.2 fmr
0	42.9 fmr	19.8 fmr
0	96	41
0	127	40
-17	<LOD	24 fmr
-27	24.8 fmr	24.6 fmr
-31	137 fmr	79 fmr
-38	71 fmr	22 qni
-49	<LOD	29 fmr
-65	<LOD	<LOD
-85	<LOD	16qni

LOD – level of detection

fmr – failed mass ratio

qni – qualifier not integrated

Appendix 4:

Screen Results “Error” message Sent for Confirmation

EtG Screen	EtG Confirmation	EtS Confirmation
Error	> 10000	1490
Error	18.5 fmr	488
Error	76.6 fmr	160
Error	67.2 fmr	72
Error	34.4 fmr	29.7
Error	<LOD	24.5
Error	<LOD	7.72 fmr
Error	<LOD	<LOD
Error	6.43 fmr	<LOD
Error	78.7 fmr	41.5 fmr
Error	58.1 fmr	22.4 fmr
Error	51 fmr	20.4 fmr
Error	42.6 fmr	55.3 fmr
Error	31.3 fmr	63.8 fmr
Error	27.2 fmr	50.1 fmr
Error	25.3 fmr	67.1 fmr
Error	23.3 fmr	42.6 fmr
Error	21 fmr	33 fmr
Error	18.1 fmr	35.1 fmr
Error	14 fmr	9.4 fmr
Error	3.6 fmr	385 fmr

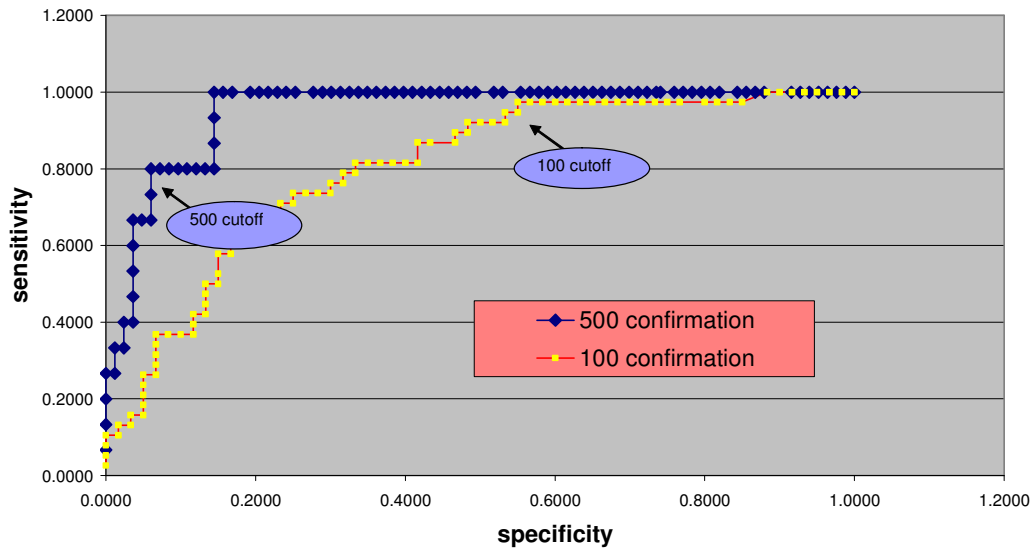
LOD – level of detection

fmr – failed mass ratio

Appendix 5:

ROC curve using 98 samples

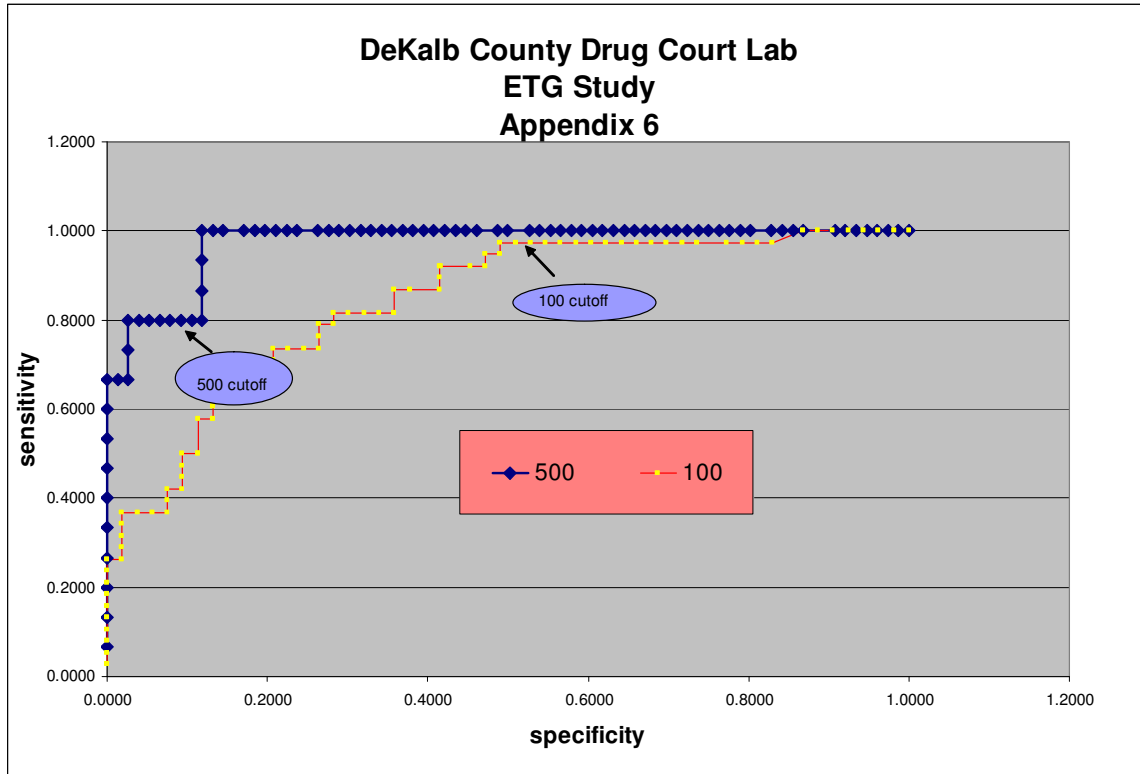
**DeKalb County Drug Court Laboratory
ETG Study
Appendix 5**



*Note: The highest level of accuracy for the ROC curve is found at the closest plot to the upper left corner.

Appendix 6:

ROC curve using 91 samples



*Note: The highest level of accuracy for the ROC curve is found at the closest plot to the upper left corner.

Appendix 7:

Bayesian Analyses

Bayesian Analysis at 500 ng/mL cut-off

N=98	EtG present	No EtG present
Positive Test	TP: 11	FP: 5
Negative Test	FN: 4	TN: 78

N=91	EtG present	No EtG present
Positive Test	TP: 11	FP: 2
Negative Test	FN: 4	TN: 74

500 Cut-off	*Sensitivity	*Specificity	*Positive Predictive Value	*Negative Predictive Value
98 Samples	0.73333	0.9398	0.6875	0.9512
91 Samples	0.73333	0.9737	0.8462	0.9487

*Sensitivity = $TP/(TP+FN)$

*Specificity = $TN/(FP+TN)$

*Positive Predictive Value = $TP/(TP+FP)$

*Negative Predictive Value = $TN/(FN+TN)$

Bayesian Analysis at 100 ng/mL cut-off

N=98	EtG present	No EtG present
Positive Test	TP: 37	FP: 33
Negative Test	FN: 1	TN: 27

N=91	EtG present	No EtG present
Positive Test	TP: 37	FP: 26
Negative Test	FN: 1	TN: 27

100 Cutoff	*Sensitivity	*Specificity	*Positive Predictive Value	*Negative Predictive Value
98 Samples	0.9737	0.45	0.5286	0.9643
91 Samples	0.9737	0.5094	0.5873	0.9643

*Sensitivity = $TP/(TP+FN)$

*Specificity = $TN/(FP+TN)$

*Positive Predictive Value = $TP/(TP+FP)$

*Negative Predictive Value = $TN/(FN+TN)$