D. Substance Abuse/Intoxications

Nicotine

Cope G, Nayyar P, Holder R, Gibbons J, Bunce R. A simple near-patient test for nicotine and its metabolites in urine to assess smoking habit. Clinica Chimica Acta 1996;256(2):135-49

Jacob P 3rd, Yu L, Shulgin AT, Benowitz NL. Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes. American Journal of Public Health 1999;89(5):731-6

James H, Tizabi Y, Taylor R. Rapid method for the simultaneous measurement of nicotine and cotinine in urine and serum by gas chromatography-mass spectrometery. Journal of Chromatography B 1998;708:87-93

Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C, Saloojee Y. Comparison of tests used to distinguish smokers from nonsmokers. American Journal of Public Health 1987;77(11):1435-8

Klein J, Koren G. Hair analysis – a biological marker for passive smoking in pregnancy and childhood. Human and Experimental Toxicology 1999;18(4):279-82

Leischow SJ, Merikle EP, Cook G, Newman R, Muramoto M. An evaluation of NicCheck I: a dipstick method for analyzing nicotine and its metabolites. Addictive Behaviors 1999;24(1):145-8

Oddoze C, Pauli AM, Pastor J. Rapid and sensitive high-performance liquid chromatographic determination of nicotine and cotinine in nonsmoker human and rat urines. Journal of Chromatography B 1998;708:95-101

Author:	**Cope G, Nayyar P, Holder R, Gibbons J, Bunce R**
Title:	**A simple near-patient test for nicotine and its metabolites in urine to assess smoking habit**
Source:	**Clinica Chimica Acta 1996;256(2):135-49**

The aim of this study was to incorporate a colorimetric assay for nicotine metabolites into a simple to use, inexpensive, disposable device, that could be used to test urine to assess smoking habit in extra-laboratory situations. By changing color, the device can give a qualitative assessment of tobacco use, but quantitative measurement of nicotine metabolite concentration in urine can also be made. Two observers examined the urine of 62 smokers and 18 non-smokers, resulting in a sensitivity of 89% and specificity of 100%. The authors observed that determination of color by eye is subjective and prone to misinterpretation; uncertainty can be removed by classifying only those samples that have a distinct color change as positive. A desired attribute of any biochemical test for smoking is a direct relationship between the result and actual cigarette use. There was a significant correlation between test results and reported consumption. The authors believe that despite its imperfect degree of sensitivity, this method does have the advantage of being an extra-laboratory test.

Author:	**Jacob P 3rd, Yu L, Shulgin AT, Benowitz NL**
Title:	**Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes**
Source:	**American Journal of Public Health 1999;89(5):731-6**

This study reports on the levels of various tobacco alkaloids in commercial tobacco products and in the urine of persons using such products. Levels of these alkaloids were compared with nicotine intake to assess their usefulness as markers of tobacco use or exposure. 34 men participated in this study, of which 12 smoke cigarettes, 9 use smokeless tobacco, 5 smoke pipes, and 8 smoke cigars. For the first three days, the men smoked like they usually do, but on the last two days, they did not smoke at all. Urine and blood samples were taken periodically. Generally, correlations of alkaloid levels with nicotine intake were good. The concentrations and excretion rates of nicotine and cotinine correlated well with nicotine intake from cigarettes as well as with nicotine from smokeless tobacco use and cigar and pipe smoking. This indicates their potential use as a biomarker of tobacco intake.

Author:	**James H, Tizabi Y, Taylor R**
Title:	**Rapid method for the simultaneous measurement of nicotine and cotinine in urine and serum by gas chromatography-mass spectrometery**
Source:	**Journal of Chromatography B 1998;708:87-93**

This paper describes a rapid and relatively simple and inexpensive method for the simultaneous determination of nicotine and cotinine in urine using gas chromatography-mass spectrometery (GC-MS). A volume of human urine or serum was spiked with solutions of various concentrations of nicotine and cotinine standards to give a concentration range of 1.25 – 100 ng/ml for nicotine and 1.25 – 1000 ng/ml for cotinine. The limit of detection of the assays was .16 ng/ml for both nicotine and cotinine; the quantitation limit for each analyte was 1.25 ng/ml. This method has several advantages over previously published ones; it uses one calibration curve for both low and high concentrations of nicotine and cotinine. The method is also sensitive, reliable, specific, rapid, and has a high degree of recovery for the analytes.

Author:	**Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C, Saloojee Y**
Title:	**Comparison of tests used to distinguish smokers from nonsmokers**
Source:	**American Journal of Public Health 1987;77(11):1435-8**

This study compares all of the markers of smoking in widespread use for their ability to correctly categorize smokers and nonsmokers. 211 subjects (52 women and 159 men) participated in the study by agreeing to fill out a self-completion questionnaire giving details of smoking habit and to give samples of blood, expired air, saliva, and urine. 188 (89%) reported smoking cigarettes at some time, and 90 (43%) said they currently smoke cigarettes, pipes, or cigars. Gas chromatography determined the concentration of nicotine and cotinine in plasma, saliva, and urine. Caronoxyhaemoglobin concentrations were measured with an IL282 CO-Oximeter. With the exception of urinary thiocyanate, all of the tests performed reasonably well in identifying smokers; cotinine performed better than nicotine. Sensitivities were higher for measures based on nicotine than for CO or thiocyanate. Specificity was less satisfactory, because 15-20% of claimed nonsmokers were classified as smokers by each test. In summary, cotinine provides the best discrimination and must be the marker of choice for situations where accuracy is paramount. Noninvasive specimens of saliva or urine gave essentially the same information as blood. For routine clinical applications, expired air CO is a simple, cheap, and acceptably accurate measure.

Author:	**Klein J, Koren G**
Title:	**Hair analysis – a biological marker for passive smoking in pregnancy and childhood**
Source:	**Human and Experimental Toxicology 1999;18(4):279-82**

This study used the hair of infants and children to analyze nicotine and its metabolite cotinine as a biological marker for exposure to smoking. Hair samples from 94 mother-infant pairs were collected and each mother filled out a questionnaire about her smoking habit. Their answers categorized them as active, passive, or non-smokers. Maternal concentrations of nicotine were invariably higher than neonatal levels, but concentrations of cotinine did not differ significantly between mothers and infants. For mothers who smoke actively, hair nicotine and cotinine concentrations were significantly higher than for passive or non-smokers and there was a difference between passive and non-smokers. For infants, the nicotine concentrations in active smokers was significantly higher than in passive and non-smokers but there was no significant difference between passive and non-smokers. The authors conclude that hair analysis is an important tool for the measurement of exposure to cigarette smoke.

Author:	**Leischow SJ, Merikle EP, Cook G, Newman R, Muramoto M**
Title:	**An evaluation of NicCheck I: a dipstick method for analyzing nicotine and its metabolites**
Source:	**Addictive Behaviors 1999;24(1):145-8**

The purpose of this study was to provide information about the reliability of the test strip coding of NicCheck I and the relationship between test strip codings and other measures of nicotine consumption such as CO, cigarettes smoked per day, and urinary cotinine and nicotine. The secondary objective of the study was to determine whether a 3 or 5 color coding scheme would provide the greatest reliability. Sixty-seven Hispanic smokers were recruited from a clinical trial of the efficacy of the nicotine patch for smoking cessation. These 67 participants gave pre-quit

urine samples into which the examiners inserted the NicCheck I test strips. Two raters independently read the test strips 25-30 minutes later and compared them to the color chart. Raters agreed on 77.6% (52/67) of the samples for the five level coding and on 86.6% (58/67) of the samples of the three level codings. Reproducibility was marginal (Kappa = 0.4) for the 5-level coding and good (Kappa = 0.7) for the 3-level coding. This evaluation suggests that NicCheck I is sensitive to differences in nicotine consumption. Examiners do not know whether the false negatives were influenced by several freezings and thawings of the urine sample, inaccuracy of the technology, or for some other reason. NicCheck I may have the potential to provide a quantitative index of nicotine and its metabolites.

Author:	**Oddoze C, Pauli AM, Pastor J**
Title:	**Rapid and sensitive high-performance liquid chromatographic determination of nicotine and cotinine in nonsmoker human and rat urines**
Source:	**Journal of Chromatography B 1998;708:95-101**

This study developed a simple, fast, and sensitive reversed phase ion-paired liquid chromatographic procedure using ultra-violet detection with a single-step solid-phase extraction. The examiners analyzed nonsmokers' urine; the same urine spiked with nicotine, cotinine, and internal standard (I.S.) solutions; and a urine sample from a child exposed to environmental tobacco smoke (ETS). The present method improves a reliable procedure for the determination of cotinine levels for smokers and nonsmokers exposed to ETS. The Extrelut simplified the extraction procedure thus reducing the analysis time and improving the reproducibility. Unlike cotinine, nicotine is not an efficient biomarker – its within-day and between-day reproducibility is higher than that of cotinine, its limit of detection is not as good, and its recovery is not satisfactory. In addition, nicotine level is a reflection of recent exposure because it has a short half-life. The authors conclude that use of cotinine has "good sensitivity, recovery, reproducibility, and is suitable for a large number of applications."

Lead

Flegal AR, Smith DR. Current needs for increased accuracy and precision in measurements of low levels of lead in blood. Environmental Research 1992;58:125-33

Gordon CL, Chettle DR, Webber CE. An improved instrument for the in vivo detection of lead in bone. British Journal of Industrial Medicine 1993;50:637-41

Graziano JH. Validity of lead exposure markers in diagnosis and surveillance. Clinical Chemistry 1994;40(7):1387-90

Hu H, Rabinowitz M, Smith D. Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. Environmental Health Perspectives 1998;106(1):1-8

Jacobson BE, Lockitch G, Quigley G. Improved sample preparation for accurate determination of low concentrations of lead in whole blood by graphite furnace analysis. Clinical Chemistry 1991;37(4):515-9

Rabinowitz MB. Relating tooth and blood lead levels in children. Bulletin of Contamination and Toxicology 1995;55:853-7

Smith DR, Ilustre RP, Osterloh JD. Methodological considerations for the accurate determination of lead in human plasma and serum. American Journal of Industrial Medicine 1998;33:430-8

Author:	**Flegal AR, Smith DR**
Title:	**Current needs for increased accuracy and precision in measurements of low levels of lead in blood**
Source:	**Environmental Research 1992;58:125-33**

These authors make a claim for more accurate and precise instruments to measure low levels of lead in blood, because currently methods can only detect blood levels > 0.5 (mirco)M. Below this level, measurements cannot be made and so the threshold of subclinical lead toxicity in humans cannot be established. It should be noted that extraneous sources may contaminate the blood sample, leading to a higher measurement. For instance, lead contamination in blood collection tubes, though routinely dismissed as insignificant or not detectable, may be a primary source of contamination. Analytical reagents and cleaned labware may also contaminate samples. Modifications in measurement, lab techniques, and equipment make comparisons difficult. Another problem results from imprecise analytical methodologies that can adequately identify persons with elevated exposure but not with lower environmental exposures. The author concludes by stating "current analytical procedures are not adequate to measure the 'true' low PbB levels of environmentally exposed individuals."

Author:	**Gordon CL, Chettle DR, Webber CE**
Title:	**An improved instrument for the *in vivo* detection of lead in bone**
Source:	**British Journal of Industrial Medicine 1993;50:637-41**

This paper describes an upgraded 109Cd system that uses a point source centered on the face of and shielded from a high purity germanium (HPGe) detector for the *in vivo* detection of lead in bone. It presents design differences between this system and the improved annular source version and reports results of measurements on occupationally exposed subjects. The article's 'materials and methods' section fully explains the instrument. Just about two thirds of the population variation in lead concentrations in bone is due to measurement uncertainty with biological variation between subjects accounting for the remainder. Once the precision is improved, researchers can place greater confidence on the results they obtain. The authors conclude that 'this work has shown that the use of a point source and a large area detection improves the precision of K X-ray fluorescence excitation measurements of lead concentration in bone." This research team was able to improve the minimum detectable limit for the instrument by almost a factor of two and therefore increase the confidence with which raised concentrations of tibia lead can be identified.

Author:	**Graziano JH**
Title:	**Validity of lead exposure markers in diagnosis and surveillance**
Source:	**Clinical Chemistry 1994;40(7):1387-90**

This article summarizes the research devoted to the development of biomarkers of exposure to lead. Indirect methods include the anlaysis of precursors and enzymes of a biosynthetic pathway (heme) in blood and urine. Measurement in blood, bone, tooth, chelatable, and stable isotope dilution comprise direct methods. The strengths and weaknesses of each method for diagnosis or epidemiological research depend on the objective of the study.

Heme pathways have been used a s a source of biomarkers for lead exposure because of the ease of sampling blood. People have studied erythrocyte aminolevulinic acid dehydratase,

aminolevulinic acid, and erythrocyte protoporphyrin. All of these methods are actually markers of the effects of lead on heme biosynthesis.

Calcified tissues provide another source of information about lead exposure. Lead exposure can be measured in deciduous teeth, but teeth are not usually available for analysis. X-ray fluorescence can make a direct *in vivo* measurement of lead in bone, and epidemiological studies have used this method. However, it is expensive, involves low dose radiation exposure, takes time, is not widely available, and cannot reliably measure low concentrations like those of environmentally exposed persons.

Mass spectrometry of stable lead isotopes in whole blood allows one to use the stable isotope dilution technique as a forensic tool for experimental and clinical studies. The cost and high degree of technical expertise necessary to conduct these analyses limit their use.
Measurement of lead elimination in urine after a single injection of the chelating agent CaNa2 EDTA can also serve as a biomarker, but this method has many limitations including the requirement of drugs, nursing care, and money.

Blood lead is most useful as a marker of recent lead exposure but it cannot measure cumulative lifetime exposure. All of the major studies of pregnancy outcomes, cognitive function in children, and renal function in adults have used blood lead as the marker.

Blood lead has become the most widely used and informative biomarker of lead exposure and is currently the method of choice.

Author: **Hu H, Rabinowitz M, Smith D**
Title: **Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms**
Source: **Environmental Health Perspectives 1998;106(1):1-8**

This review discusses the measurement of skeletal lead using X-ray fluorescence (XRF), a rapid, safe, accurate, and relatively precise method of measuring lead in bone. The authors believe that the most important determinant of lead toxicity is the cumulative measure of lead dose rather than the current blood lead level. Two types of XRF exist – LXRF and KXFR; this paper focuses on KXRF because it has been more widely used and has proven to measure cumulative lead dose in epidemiological studies. Despite the understanding that bone lead levels reflect lead exposure, researchers have given very little attention to conceptualizing this paradigm. Clarifying these paradigms will make it possible to make hypotheses about bone lead accumulation and release, select bone sites for measurement, and design epidemiological studies. The authors believe lead in the skeleton has two roles as a marker of chronic lead toxicity in adults. First, bones serve as repositories for lead and serve as a proxy for cumulative lead dose. Second, the skeleton is an endogenous source of lead. The authors provide numerous examples from the literature to support their two hypotheses. If the skeleton is a source of lead, the authors state that “factors that influence bone turnover and therefore bone lead mobilization may modify the toxicity of lead that has accumulated in the skeleton over time.” Making separate measurements of trabecular and cortical bone lead and incorporating markers of bone turnover and measurements of bone mineral status may help establish the relative importance of one of the two paradigms.

Author:	**Jacobson BE, Lockitch G, Quigley G**
Title:	**Improved sample preparation for accurate determination of low concentrations of lead in whole blood by graphite furnace analysis**
Source:	**Clinical Chemistry 1991;37(4):515-9**

Precision and accuracy in low concentration blood lead measurement has improved greatly. Zeeman graphite-furnace atomic absorption spectrometry (GFAAS) can directly determine lead in whole blood. This article describes a sample preparation of GFAAS that yielded within-run CVs of 3.2%, 1.8%, and 1.4% at lead concentrations of .25, 1.98, and 3.76 micro mol/L and between-run CVs of 7.3%, 2.9%, and 2.2%. Analytical recovery data show that the results compare favorably with target values established for both external quality control programs. The reproducibility/accuracy score in the Quebec Toxicology program was 96%, putting the lab in second place out of 66 participating labs. Problems associated with direct analysis of whole blood by GFAAS include imprecise dispensing of whole blood, effect of matrix on analyte sensitivity, incomplete recovery, poor precision, excessive accumulation of carbonaceous residues in the furnace, and the difficulty of accurate calibration. The authors conclude that there is still a critical need for greater analytical accuracy and precision at lead levels below 0.48 micro mol/L.

Author:	**Rabinowitz MB**
Title:	**Relating tooth and blood lead levels in children**
Source:	**Bulletin of Contamination and Toxicology 1995;55:853-7**

Blood is used much more often than teeth in assessing lead exposure, but teeth are more anatomically complex and results obtained from samples of whole shed teeth, of only the crown, or of only the dentine are nearly identical. Few studies have related blood lead levels to teeth using the same children. The author found considerable consistency among these studies; a straight line fits the data for the eight studies reviewed in this article. There is still uncertainty about the age at which the blood lead is best preserved in the shed tooth. In some cases, the blood lead levels of a child do not change with age, but when it does, it appears that the shed dentine reflects blood lead levels fairly recently, as opposed to many years earlier. In comparison, in adult teeth, lead accumulates for many years and the observations made about primary teeth do not hold true. The author believes that the eight studies demonstrate the usefulness of shed teeth as a biomarker for past lead exposure.

Author:	**Smith DR, Ilustre RP, Osterloh JD**
Title:	**Methodological considerations for the accurate determination of lead in human plasma and serum**
Source:	**American Journal of Industrial Medicine 1998;33:430-8**

This article raises some important issues related to the accurate measurement of lead in human plasma and serum. Venous blood was obtained by two sampling methods, routine and ultraclean, from three subjects without history of lead exposure. After 10 minutes of centrifugation, the authors analyzed the samples using inductively coupled plasma-high-resolution mass spectrometry (ICP-MS). The study compared the ultraclean serum collection method with the plasma collection method, the effect of standing time prior to centrifugation on lead

concentration, and the use of commercial heparinized vacutainer tubes versus an ultraclean plasma sampling method that used a low-lead heparin anticoagulant.

Whole blood Pb lead	Ultraclean serum Pb level	Vacutainer plasma PB level
1.8 micro g/dl	.40%	1.7%
2.0 micro g/dl	.30%	1.5%
2.7 micro g/dl	.48%	2.4%

Whole blood standing times of 15, 40, and 70 minutes before centrifugation resulted in increased ultraclean serum-lead levels of .21%, .81%, and 1.2%, but it had no effect on plasma lead levels using vacutainers. These data indicated that substantial increases in serum-lead levels may occur with increasing whole blood clotting duration, and these increases in serum-lead are paralleled by increases in serum lead of a much smaller magnitude. Hemolysis contributed significantly to both plasma lead and serum lead levels. In summary, the authors demonstrated that plasma-lead and serum lead levels are dependent upon methodological processing techniques including lead contamination control, redistribution due to EDTA anticoagulant, hemolysis, and time dependency in simple processing.

Other Substance Abuse

Buchan BJ, Walsh JM, Leaverton PE. Evaluation of the accuracy of on-site multi-analyte drug testing devices in the determination of the prevalence of illicit drugs in drivers. Journal of Forensic Sciences 1998;43(2):395-9

Crouch DJ, Cheever ML, Andrenyak DM, Kuntz DJ, Loughmiller DL. A comparison of ONTRAK TESTCUP, Abuscreen ONTRAK, Abuscreen ONLINE, and GC/MS urinalysis test results. Journal of Forensic Sciences 1998;43(1):35-40

Crouch DJ, Frank JF, Farrell LJ, Karsch HM, Klaunig JE. A multiple-site laboratory evaluation of three on-site urinalysis drug-testing devices. Journal of Analytical Toxicology 1998;22(6):493-502

Ferrara SD, Tedeschi L, Frison G, Brusini G, Castagna F, Bernardelli B, Soregaroli D. Drugs-of-abuse testing the urine: statistical approach and experimental comparison of immunochemical and chromatographic techniques. Journal of Analytical Toxicology 1994;18:278-91

Moore C, Deitermann D, Lewis D, Feeley B, Niedbala RS. The detection of cocaine in hair specimens using micro-plate enzyme immunoassay. Journal of Forensic Sciences 1999;44(3):609-12

Smith FP, Lora-Tamayo C, Carvajal R, Caddy B, Tagliaro F. Assessment of an automated immunoassay based on kinetic interaction of microparticles in solution for determination of opiates and cocaine metabolite in urine. Annals of Clinical Biochemistry 1997;34(pt1):81-4

Towt J, Tsai SCJ, Hernandez MR, Klimov AD, Kravec CV, Rouse SL, Subuhi HS, Twarowska B, Salamone SJ. ONTRAK TESTCUP: a novel, on-site, nulti-analyte screen for the detection of abused drugs. Journal of Analytical Toxicology 1995;19:504-10

Wennig R, Moeller MR, Haguenoer JM, Marocchi A, Zoppi F, Smith BL, de la Torre R, Carstensen CA, Goerlach-Graw A, Schaeffler J, Leinberger RDevelopment and evaluation of immunochromatographic rapid tests for screening of cannabinoids, cocaine, and opiates in urine. Journal of Analytical Toxicology 1998;22(2):148-55

Author:	**Buchan BJ, Walsh JM, Leaverton PE**
Title:	**Evaluation of the accuracy of on-site multi-analyte drug testing devices in the determination of the prevalence of illicit drugs in drivers**
Source:	**Journal of Forensic Sciences 1998;43(2):395-9**

The principle goal of this research was to conduct a field evaluation of 'on-site' multi-analyte drug testing devices to determine the most accurate, efficient, and cost-effective device available for the purpose of rapidly detecting drivers under the influence of drugs. Voluntary and legal urine specimens were collected from 305 persons placed under arrest for suspicion of DUI; 303 specimens contained a sufficient amount of urine for testing. The researchers compared the following four tests:

1. Triage – phencyclidine (PCP), benzodiazepines (BZO), cocaine (COC), amphetamine (AMP), marijuana metabolites (THC), opiates (OPI), and barbituates (BAR)
2. Abu-Sign – THC, OPI, COC, AMP/MET
3. OnTraK – THC, COC, morphine (MOR)
4. TesTcup – THC, COC, MOR

Immunoassay and gas chromatography mass spectrometry were used as the gold standard comparison test. Comparison of the four on-site kits indicated some differences in ease of handing, time to conduct the test, specimen handling, reagent mixing, and readability of results. Four evaluators independently ranked the four tests on a scale of 1 = poor to 4 = excellent. The evaluators preferred Abu-Sign and TesTcup because they do not require reagent mixing/handling. The Abu-Sign gives results in five minutes; OnTrak has the most cumbersome and time-consuming procedure.

Kit	Marijuana				Cocaine				Opiates			
	Sen	Spec	PPV	NPV	Sen	Spec	PPV	NPV	Sen	Spec	PPV	NPV
Triage	91.7	99.6	97.8	98.4	85.0	99.6	97.1	97.8	100	99.7	66.7	100
Abu-Sign	100	92.9	72.7	100	100	97.7	86.9	100	100	100	100	100
TesTcup	91.5	98.4	91.5	98.4	95.0	97.7	86.4	99.2	100	100	100	100
OnTrak	82.9	98.0	88.6	96.9	82.5	99.6	97.0	97.4	100	100	100	100

Overall, all four test kits performed will, work well as screening devices, and can be used by non-medical persons.

Author:	**Crouch DJ, Cheever ML, Andrenyak DM, Kuntz DJ, Loughmiller DL**
Title:	**A comparison of ONTRAK TESTCUP, Abuscreen ONTRAK, Abuscreen ONLINE, and GC/MS urinalysis test results**
Source:	**Journal of Forensic Sciences 1998;43(1):35-40**

This study compared results from two drug testing kits – ONTRAK, TESTCUP, and Abuscreen ONTRAK – with results from lab based immunoassay and gas chromatography mass spectrometry (GC/MS) for cocaine, morphine, and cannabinoids. Researchers studied 250 negative samples and 100 presumed positive samples each for cocaine/metabolites, opiates, and cannabinoids.

	Opiate Positive		Cocaine Positive		THC-COOH positive	
	Discrepancy	% agreement	Discrepancy	% agreement	Discrepancy	% agreement
TestCup	100/100	100%	98/100	98%	92/100	92%
OnTrak	100/100	100%	91/100	91%	89/100	89%

In this study, TESTCUP had 100% agreement with GC/MS and a >99% agreement with ONLINE when testing negative samples. When testing positive samples, the most agreement between TESTCUP and ONLINE results was with opiate positive category samples. ONTRAK had 100% agreement with both GC/MS and ONLINE when testing negative samples. When testing opiate positive samples, the most agreement between ONTRAK and ONLINE results was found. The performance of both TESTCUP and ONTRAK was improved when the urine contained a verified concentration of the drug in excess of the assay's published cutoff. ONTRAK is portable, easily performed, and can test single drug classes. TESTCUP is self-contained, tests for most common drugs, and does not require a technician. The sensitivity and specificity for TESTCUP analysis of QC samples was 100% for BZE, opiates and THC-COOH. The calculated ONTRAK sensitivities were 97.7%, 100%, and 100% for BZE, opiates, and THC-COOH and the specificity was 100 for the three tested drugs. The authors conclude that TESTCUP and Ontrak are effective urinalysis drug-testing techniques.

Author: **Crouch DJ, Frank JF, Farrell LJ, Karsch HM, Klaunig JE**
Title: **A multiple-site laboratory evaluation of three on-site urinalysis drug-testing devices**
Source: **Journal of Analytical Toxicology 1998;22(6):493-502**

This study had three separate drug-testing labs evaluate three different devices – EZSCREEN, ONTRAK, and TRIAGE – against results from Syva EMIT immunoassay and gas chromatography spectrometry (GC-MS) for amphetamines, benzodiazepines, cocaine, cannabinoids, and opiates. Each lab selected 20 urine samples that tested positive for a single drug and 20 that tested negative. Qualitative and quantitative GC-MS confirmed that the positive samples actually contained the target drug and that the negative samples did not. False positives were rare; both EZSCREEN and TRIAGE had two false positive results. Cross-reactivity from samples containing GC-MS verified high concentrations of alternate drugs was also rare. Comparing on-site test device results with those of EMIT for samples with drug concentrations near the reporting cutoff proved difficult, because it requires thorough knowledge of the performance of each device and an investigation of each discrepant result. All three methods effectively detect drugs of abuse in urine samples and gave results similar to the gold standard – Syva Emit immunoassay and GC-MS. Furthermore, different labs cans produce the same results using the same device. The authors caution that "comparing OTD (on-site drug-testing devices) results with laboratory based immunoassay or GC-MS results should be done cautiously and with full disclosure of the data to avoid potential misinterpretation."

Author:	**Ferrara SD, Tedeschi L, Frison G, Brusini G, Castagna F, Bernardelli B, Soregaroli D**
Title:	**Drugs-of-abuse testing the urine: statistical approach and experimental comparison of immunochemical and chromatographic techniques**
Source:	**Journal of Analytical Toxicology 1994;18:278-91**

This study compared the following six immunochemical techniques EIA-EMIT, EZSCREEN, FPIA-ADx, RIA-Coat-A-Count, LI-Abuscreen ONTRAK, and CBI-Triage and three chromatographic techniques TLC-ToxiLab, HPLC, and HPLC-REMEDi drug profiling system against the gold standard gas chromatography mass spectrometry (GC-MS). All testing methods can detect amphetamines, barbituates, benzodiazephines, cannabinoids, cocaine, methadone, and opiates in the urine of persons who use these drugs. 635 urine samples were taken from patients hospitalized for acute intoxication (20.1%), people with psychoactive drug addiction (60.4%), subjects examined for 'fitness for duty' (15.6%), and persons who died of toxic causes (3.9%). The article lists the sensitivity, specificity, false positive rate, and false negative rate for all ten techniques studied and for all seven target drugs. It also gives results for positive and negative predictive values for the ten techniques and seven target drugs. The authors believe that "the preliminary degree of assessment of the capability and reliability of analytical techniques in selecting positive and negative samples must first be based on sensitivity, specificity, false positive rates, and false negative rates." Furthermore, the cumulative predictive values extrapolated from this study confirm that the combination of immunochemical and chromatographic techniques gives better guarantees regarding the degree of reliability of the results.

Author:	**Moore C, Deitermann D, Lewis D, Feeley B, Niedbala RS**
Title:	**The detection of cocaine in hair specimens using micro-plate enzyme immunoassay**
Source:	**Journal of Forensic Sciences 1999;44(3):609-12**

The present study describes the detection of cocaine in human hair using a commercially available micro-plate enzyme immunoassay. Hair has several advantages over urine as a source of information about drug use. Long hair can give evidence of historical use and external exposure, and it is easy to collect and store. The sample size (100, 50, 25, 10 MircoL) and initial incubation time (15, 30, 45, and 60 minutes) were the major variables in the development of this assay. The samples were analyzed by fluorescence polarization immunoassay (FPA) and microplate immunoassay (EIA); gas chromatography mass spectrometry was used as the gold standard.

	Sensitivity	Specificity	Efficiency
FPIA	67.8	80.5	77.1
Micro-plate EIA	75	97.4	91.4

FPIA is designed for urinalysis so is targeted primarily at bezoylecgonine. Since concentrations of bezoylecgonine in hair tend to be lower than that of parent cocaine, any immunoassay that specifically targets cocaine should be more sensitive for hair analysis. Microplate EIA had a greater sensitivity, specificity, and efficiency than FPIA for the detection of cocaine in hair.

Author:	**Smith FP, Lora-Tamayo C, Carvajal R, Caddy B, Tagliaro F**
Title:	**Assessment of an automated immunoassay based on kinetic interaction of microparticles in solution for determination of opiates and cocaine metabolite in urine**
Source:	**Annals of Clinical Biochemistry 1997;34(pt1):81-4**

This study assessed the performance of the recently developed commercial kit ONLINE using kinetic interaction of microparticles in solution (KIMS) technology for the analysis of opiates and cocaine metabolite in urine against the most widely used EIA analysis and specific chromatographic methods. For the GC method, the average analytical recoveries were 90% for morphine, 93% for codeine, 80% for 6 monoacetylmorphine, and 98% for cocaine. When morphine and BE concentrations were less then the detection limits of other methods, GC/MS was adopted. The within-day precision ranged from 1.4 to 4.7% for morphine and from 4.2 to 4.8% for BE. The repeatability of the standards over one month was 1.0–3.3% for opiates and 1.7-5.1% for BE and thus allows measurements to continue over thirty days without re-calibration. The KIMS technology used by ONLINE has many advantages for testing drugs of abuse.

Author:	**Towt J, Tsai SCJ, Hernandez MR, Klimov AD, Kravec CV, Rouse SL, Subuhi HS, Twarowska B, Salamone SJ**
Title:	**ONTRAK TESTCUP: a novel, on-site, multi-analyte screen for the detection of abused drugs**
Source:	**Journal of Analytical Toxicology 1995;19:504-10**

This paper describes a novel, rapid, on-site, multi-analyte screen named ONTRAK TESTCUP that detects benzoylecgonine (cocaine), morphine, and cannabinoids in urine. Briefly, a sample is collected in the TESTCUP, a lid is placed on it, and a chamber at the top of the cup is filled with urine by inverting the cup for 5 seconds. When the urine contacts the immunochromatographic strips, the assays are developed. Results appear 3-5 minutes later; a colored bar at the detection window indicates a negative result and the absence of color indicates a positive result. If positive, the same device can be used for GC-MS. For urine controls containing drug at 50% of its cutoff concentration, the results were greater than or equal to 96, 98, and 96% negative for cocaine, morphine, and THC. For urine controls containing drug at 120% of its cutoff concentration, the results were greater than or equal to 97, 100, and 98% positive for the same three drugs. There was 100% agreement between samples prescreened positive by GC-MS and positive by TESTCUP for all three assays. There was 100% agreement between TESTCUP and ONTRAK results and between TESTCUP and ONLINE results when testing clinical samples positive and negative for cocaine or THC. Testcup is easy to use, reliable, requires no reagent preparation or handling, and produces results in less than five minutes. Furthermore, positive samples can be kept in the Testcup and sent to a lab for further study.

Author:	**Wennig R, Moeller MR, Haguenoer JM, Marocchi A, Zoppi F, Smith BL, de la Torre R, Carstensen CA, Goerlach-Graw A, Schaeffler J, Leinberger R**
Title:	**Development and evaluation of immunochromatographic rapid tests for screening of cannabinoids, cocaine, and opiates in urine**
Source:	**Journal of Analytical Toxicology 1998;22(2):148-55**

This paper describes FRONTLINE rapid test for the detection of drugs of abuse in urine and compares its results to FPIA and EMIT assays. FRONTLINE screens for cannabinoids, cocaine, opiates, and amphetamines using gold-labeled immunochromatography technology. Six testing sites evaluated this new technique. The rapid tests must be dipped into the urine sample for 3-5 seconds, placed horizontally, and read two minutes later. Reaction colors have been shown to be stable for up to ten minutes. Color results indicate a negative, single positive, or double positive color block. For cannibinoids, sensitivities and specificities were 97% or better for comparisons with both FPIA and EMIT. For cocaine, the test had a sensitivity of 100% compared to FPIA and EMIT but only had a specificity of 91% because of cross-reactivity with metabolites of methadone and clozapine. For opiates, sensitivity and specificity were 99% or better. The FRONTLINE rapid test gave very reliable results in this multi-site study and should be recommended for the detection of drugs of abuse, especially when an immediate result is needed.