THE POWER OF DRUG TESTING

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> One of the most important works in the Hippocratic Collection is Airs, Waters, and Places (5th century B.C). Hippocrates is breathtaking in his vision in this work when, instead of ascribing diseases to divine origin, discusses their environmental causes. He proposes that a physician consider a town's weather, drinking water, and site along the paths of favorable winds to help ascertain the general health of citizens.

ABSTRACT: Human beings are the most curious of all organisms. Our tendency is to inspect, dissect and analyze anything new in our world in an attempt to understand it. Over many centuries, we have developed the unique ability to identify and reproduce natural chemical substances found in the environment. As our knowledge of chemistry advanced, we learned how to make new synthetic drugs that heretofore did not exist in nature. As part of these experiments, humans also learned about uses and abuses of new drugs. Widespread experimentation and abuse of drugs has become commonplace in modern society, but excessive drug abuse has also become a threat to our existence. As a result, scientists in the twentieth-century developed methods for detection, monitoring, and treatment of drug abuse. Scientific and technological advances in the forties, fifties and sixties set the stage for development of highly sophisticated analytical methods that could be used for drug testing. Widespread implementation of workplace drug testing programs in the US in the seventies and eighties highlighted public awareness of the drug abuse problems that were occurring in their communities. Continued development of drug testing methodologies in the eighties and nineties provided health specialists with the analytical tools needed for diagnosis and treatment of drug abuse. This evolution and development of drug testing technology has a fascinating history that is intertwined with and often resulting from simultaneously occurring historical events. Frequently, society's concerns with war, disease, environmental pollution, and natural or human-made calamities were translated into meaningful public support of science. Increased legislative actions and financial resources were directed toward advancing scientific endeavors that were deemed worthy by the general public. Major educational and scientific directives were begun to protect the environment, explore space, develop national defense systems, and cure diseases. Much of the drug testing technology now employed evolved out of these publicly supported efforts. As the current century draws to a close, drug epidemics and the associated spread of diseases continues to threaten the health and financial stability of many nations. Clearly, many countries will continue to use existing technology and implement new drug testing programs. Access to newer and more reliable drug testing technologies will enhance these efforts, but still may not guarantee their success. It shall be the responsibility of each country to utilize this technology wisely. Drug testing is frequently performed on individuals to determine if they have abused drugs in the recent past. The process of drug testing invokes great power to those who are in the position of interpreting test results. Liberty, employment and financial gain may be decided on the basis of drug test results. The unique power of drug testing is exemplified by the millions of decisions that are now made annually in the US regarding an individual's suitability for employment. These decisions are frequently based solely on drug testing results. Other countries throughout the world now use drug testing in forensic investigations, in drugged driving cases and in many other applications. This article provides a brief examination of how this powerful technology has evolved against a background of social strife, upheaval, triumph and change.

KEY WORDS: Drug testing; Historical developments of drug testing; Power of identification.

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MANKIND'S DISCOVERY OF PSYCHOACTIVE SUBSTANCES

In the broadest sense, drug testing is an extension of mankind's curiosity about his environment. Curiosity seems to be at the core of the human psyche. Man's very nature is to explore, question, dissect and test anything new in his world. Much of humankind's explorative effort has been directed toward the environment. Exploration of the environment naturally took the form of touching and tasting. Certainly, humans have been experimenting with drugs in the form of natural products since the beginnings of time. Many highly potent substances have been identified through these early explorative efforts. As early as the 16th century B.C., opium was listed in the giant Egyptian reference work called the Ebers Papyrus. Human experience with cocaine and marijuana dates back at least 5,000 years. Cocaine was believed by the Incas to be of divine origin, consequently they reserved its use to royalty. *Cannabis* was cultivated widely in Europe and Asia for a variety of purposes including manufacture of rope and paint. *Cannabis* is described in Chinese literature in 2737 B.C. and was used very early as an analgesic in surgery. Peyote's first documented use was by the Mexican Indians from the Rio Grande Valley in Northeast Mexico at the time of the Spanish invasion. Tobacco appears to have originated thousands of years ago in North America; its use had spread throughout Europe, Asia and Africa by the 17th century.

THE EMERGENCE OF ABUSABLE DRUGS IN SOCIETY

It was not until the 17th and 18th century that chemists were able to refine their knowledge and skills to the point where pure drugs could be isolated, studied and their molecular structure elucidated. With these advances in chemical expertise came the ability to create powerful drug extracts from natural sources as well as synthetic drugs that did not exist in nature. Stimulants such as the amphetamines and depressants such as the barbiturates were synthesized in the late 1800's. The benzodiazepines and LSD followed in the early 1900's. This wealth of new psychoactive drugs resulted in an era of experimentation and abuse by the general populace. Many of the new drug products had powerful effects with addictive potential never before experienced. All too frequently, their use was enthusiastically promoted by entrepreneurs as well as the medical profession as a panacea for cures of common ailments and even deadly diseases. Many of these drugs became the subject of widespread abuse and restrictive measures were taken by governments to curtail their spread and abuse.

HISTORICAL DEVELOPMENTS AND ANALYTICAL TECHNOLOGY IN THE 1940'S

An epidemic of drug use combined with the spread of diseases in this century led to renewed efforts by governments to restrict drug use. Government leaders turned to scientists to provide the technology to combat the abuse of licit and illicit drugs. Chemical methodologies were sought that provided objective measures of drug use. Rapid, sensitive methods of drug detection were needed for use in the diagnosis, treatment and prevention of drug abuse. The technology needed for widespread drug testing had been developing over the forties and fifties. A transition had occurred in the forties from "wet" chemical methods to an era of instrumentation. Post WWII lead to massive consumerism, while spin-offs from the continuing postwar military research engendered discovery and development of discoveries such as paper chromatography, nuclear magnetic resonance and the transistor. The forties ended with some outstanding instrumental accomplishments such as the determination of the structure of penicillin that was aided for the first time by an electronic computer.

HISTORICAL DEVELOPMENTS AND ANALYTICAL TECHNOLOGY IN THE 1950'S

The fifties saw the beginnings of the Cold War, loss of China to communism and the beginnings of an era of analytical instrumentation. Gas chromatography was introduced during this decade as well as the versatile Bausch & Lomb Spectronic 20 spectrophotometer. Other major instruments introduced at this time included the reliable "poor man's" infrared instrument (IR-6 by Beckman), radioimmunoassay, and the first coupling of gas chromatography with mass spectrometry (GC/MS). The launching of Sputnik by the Soviet Union in 1957 came like an electrifying shock to the United States who considered itself in the technological lead. The following year became a banner year in the United States for support of science in general and for the development of analytical instrumentation. The National Defense Educational Act was passed to support education of new scientists in critical areas, and the National Aeronautics and Space Administration (NASA) was established. The infamous Delaney Clause of the Food, Drugs and Cosmetics Act was also passed in 1958. The Delaney Clause introduced a new testing requirement that dictated no "detectable" carcinogens would be allowed in food products. No definitions were set forth for limits of detection leading instrument manufacturers into a major struggle to extend the detection limits of their analytical systems to lower and lower concentrations.

HISTORICAL DEVELOPMENTS AND ANALYTICAL TECHNOLOGY IN THE 1960'S

A panoply of new chemicals needed to be measured in forensics, medicine, environment, agriculture, and the petrochemical industry. New assay technologies were needed that were structural, non-destructive, and most of all, sensitive and precise. The impetus was there for rapid developments in analytical instrumentation. The early 60's saw President Kennedy's announcement of the race to the moon by the end of the decade, the Cuban missile crisis, intensification of the Cold War, civil rights turmoil, the start of the sexual revolution, the counter-cultural revolution, Southeast Asian heroin, and addicted soldiers returning from the Vietnam war. Rachel Carson helped start a movement of her own in the environmental field with her publication, "Silent Spring", whose revelations of the dangers of pesticides in the environment awakened naturalists around the world. On the technical side, laser technology was under development, capillary columns were introduced based on the Golay patent, and the "Robot Chemist", an automated, programmable, "wet" chemistry station was advertised by Warner Chilcott. Introduction of the LKB 9000 GC/MS in 1966 was followed by the Finnigan quadrupole GC/MS in 1968. Both instruments immediately found favor in the biomedical community. By the end of the sixties, amazing advances had been made in medicine (first heart transplant, first gene isolated) and in space (Apollo 11 moon landing, July 20, 1969).

HISTORICAL DEVELOPMENTS AND ANALYTICAL TECHNOLOGY IN THE 1970'S

Many problems emerged in the seventies that impacted the development of analytical technology-pollution, energy crises, wars, famines, "Watergate", heroin epidemics, phencyclidine (in the US), and runaway inflation combined with high unemployment ("stagflation") in much of the world. High drug use by US military soldiers was viewed with national alarm as a threat to the stability of the entire nation. In response, the Department of Defense implemented a massive effort in drug testing at a time when biomedical instrumentation was flourishing through use of LC, HPLC, TLC, ion-exchange chromatography, gel electrophoresis, and gel-exclusion chromatography. However, concerns with health and the environment loomed large in the public eye leading in rapid succession to establishment of the Environmental Protection Agency and passage of the US Clean Air Act in 1970, and establishment of the National Cancer Institute in 1971.

Society's fascination with psychoactive drugs was continuing at a faster and faster pace. The PCP epidemic of the sixties had spilled over into the seventies with the realism that powerful new synthetic drugs were here to stay. Designer drugs could be synthesized readily by making small changes in molecular structures. Illicit laboratories were springing up everywhere and huge illegal profits were being made by a select few. The illicit cultivation, harvesting, and use of marijuana was flourishing. Drug abuse in sports was highlighted by the need to screen for drugs by GC in the 1972 Olympic Games. Drug testing technology was on the verge of advancing rapidly with the introduction of an automated clinical analyzer by Dupont in 1971. Syva's introduction of their EMIT (Enzyme-Multiplied Immunoassay Testing) assays and Roche's introduction of RIA (radioimmunoassay) provided the means for laboratories to screen hundreds of biological specimens in a single day. Drug testing technology received a further boost by Hewlett-Packard's introduction of the first microprocessor-controlled analytical instrument (HP-5830A GC) in 1974.

As the seventies drew to a close, analytical instrumentation continued to make remarkable gains. The Viking probes on Mars used pyrolysis GC and MS to search for life. The Apple Personal Computer (PC) and Finnigan's first liquid chromatograph-MS (LC/MS) were introduced in 1977. Finnigan's computerized GC/MS was approved by the Environmental Protection Agency (EPA) for water analysis leading Finnigan to dominate the global market at that time. Against this backdrop, there were continued signs of national and international environmental problems including "Love Canal", "Three Mile Island" and the ban by the US on chlorofluorocarbons.

HISTORICAL DEVELOPMENTS AND ANALYTICAL TECHNOLOGY IN THE 1980'S

The eighties started literally with a bang from the Mt. St. Helens blast. Concern about the environmental was translated into action in the US with the passage of the first Superfund Act in 1980. Jitters were felt throughout the 80s about over-the-counter medications when Tylenol capsules laced with cyanide resulted in several deaths in the US. Genetic testing took a giant step forward in 1983 when K. Mullis at Cetus invented PCR (Polymerase Chain Reaction). By 1984, enzyme immunoassays and fluorescent polarization immunoassays had begun to overtake RIA as the primary method for drug screening. Fears about drugs, disease and environmental pollution continued to be prominent in the news with the announcements of the Union Carbide disaster in Bhopal, India, the discovery of the AIDS virus in 1984, the Chernobyl nuclear reactor explosion in 1986 and the Valdez oil spill in 1989. Significant analytical testing advances continued to be made during the decade with the introduction of the Macintosh computer in 1984, development of matrix-assisted laser desorption ionization (MALDI) MS in 1985, and approval by the US courts of DNA fingerprint testing. By the end of the eighties, world-wide analytical sales had nearly doubled that of the previous five years to 4.9 billion USD. By this time some 10 billion chemical compounds were known and an additional 40,000 were being discovered per year.

Amazing societal changes had occurred throughout the decade as the eighties drew to a close: the Berlin Wall fell; the Soviet Union began its disintegration; the OPEC oil agreements unraveled, and the price of oil plummeted; the majority of Warsaw Pact nations had ended decades of communist rule; the AIDS epidemic was in full swing; and the first genetically-engineered organism was released into the environment. Amidst these developments, analytical instrumentation was moving toward automation and consolidation by taking advantages of existing technologies making combined analysis more feasible, e.g., GC/MS-MS, LC/MS-MS.

HISTORICAL DEVELOPMENTS AND ANALYTICAL TECHNOLOGY IN THE 1990'S

The epidemics of drug use in the US that started in the sixties, expanded in the seventies, and reached major proportions in the eighties resulted in promulgation of workplace drug testing rules for employees in the Federal government. These rules were published by the Department of Health and Human Services (DHHS) in final form in the Federal Register as the "Mandatory Guidelines for Federal Workplace Drug Testing Programs" on April 11, 1988. The Department of Transportation responded with similar rules on December 1, 1989 for implementation on January 2, 1990. So began the largest drug testing effort by any nation in recorded history. By 1996, expansion of the guidelines to other worker had brought more than 7.4 million transportation employees nationwide under the DHHS testing rules.

The nineties began with the prospects of globalization including world-wide concerns about global warming, biological and chemical warfare, subtleties of endocrine disruption, and genetic-damage to large-scale populations. Global regulatory requirements (ISO 9000, 9001, 9002, and 14000) emerged for operation in a world market. Amidst a background of global concern, Iraq invaded Kuwait, the Chunnel opened, Nelson Mandela was freed, the Savings and Loan crisis occurred in the US, and the human genome project began in 1990. By 1991, there were an estimated 10 million people in 160 countries infected with the AIDS virus. At this point, deforestation was projected at 40–50 million acres per year, Mount Pinatubo in the Philippines erupted, Iraq ignited the oil wells in Kuwait causing massive air and water pollution, and a massive cyclone in Bangladesh killed more than 100,000 people. The next year, 1992, brought approval of the North American Free Trade Agreement between the US and Mexico, hurricane Andrew caused massive destruction in the Southern United States, and a moratorium on silicon breast implants was announced by the US Food and Drug Administration (FDA). The Mosaic browser was developed by March Andreessen and Eric Bina in 1993 which more than any other development brought about the phenomenal growth of the Web and the Internet. There were only 100 web sites in 1993 compared to millions of sites, which presently exist. The lab-on-a-chip was introduced by Ciba Geigy in 1994, a microchip that performed reactions and analyzed the results by capillary electrophoresis.

Bioengineering continued to surge forward in the 1990s as a result of the technological revolution occurring in the life sciences. The first transgenic bull sired cows that produced human lactoferrin in their milk were introduced in 1994 and mice with transgenic human antibodies became available in 1995. Dolly the sheep, the first cloned animal, was born in 1996. By 1997, all 4.6 million base pairs in the E. Coli genome had been sequenced, and in 1998, the complete genome of C. elegans, a soil nematod, was mapped, the first multicellular animal. Currently, genetically engineered seeds for agriculture are being produced by the ton, but are increasing meeting backlash from environmental groups such as Greenpeace.

Environmental concern continued to grow in the nineties resulting in 160 nations reaching consensus on a global warming accord in Kyoto, Japan in 1997. Significantly, the following year, 1998, was the hottest year on record at that time.

Dissemination of scientific information leaped ahead in the nineties as a result of the Internet. By 1994, more computers were being sold than TV sets. Scientific journals such as the American Chemical Society Online, and the Journal of Pharmacology and Experimental Therapeutics, became accessible through the World Wide Web. Combinatorial chemistry became a "must have" by the pharmaceutical industry, and both Incyte and Celera Genomics announced they would independently sequence the human genome.

Analytical instrumentation advanced in the nineties with a continued push toward automation, appearance of new hyphenated methods such as MALDI-time of flight (TOF) MS, and development of proteomics (study of the interrelations and interactions of the proteins that govern almost every function of life).

DRUG TESTING IN THE NEW MILLENNIUM

Drug testing requirements in the next century will likely be very different than those currently practiced and will be influenced broadly by technologies emerging from other sources. Testing methodologies from other chemical testing programs may be equally applicable for use in drug testing. Currently in the US, two major chemical testing efforts are underway. The first program will attempt to test all industrial chemicals that are imported or produced in quantities exceeding 1 million pounds per year (high-production volume testing program, HPV program). Some 2800 chemicals will be subject to testing. The program calls for assessment of each chemical's environmental fate and pathways, ecotoxicity, and mammalian toxicity. In addition, a second testing by the EPA will focus on as many as 87,000 chemicals (pesticides, cosmetic ingredients, food additives, and other chemicals regulated under the Toxic Substances Control Act) for their potential to cause endocrine (hormone) disruption. The testing technologies to be utilized in these programs have only begun to be worked out.

Many additional public controversies will likely require the development of new testing technology. Transgenic crops and the application of discovery technology have been altering agricultural businesses world-wide. The introduction of genetically-engineered crops in agriculture in the US has been highly successful, but has met resistance in Europe. The mad cow episode shook people's confidence in government to protect the food supply. Recently, a study (*Nature* 1999, vol. 399, p. 214) showed that pollen from genetically modified corn kills monarch butterfly caterpillars. As a consequence, many European countries are calling for segregation and labeling of foodstuffs produced by genetic engineering. Limits are likely to be set on the amount of genetically engineered food that can be present and content will have to be specified. Genetic-based testing of foodstuffs will be crucial to implementation of these requirements.

Another crisis of major proportion in Bangladesh will likely require development of new testing technology. Available information indicates that more than 3 million of the approximately 5 million existing wells in Bangladesh might be contaminated with toxic levels of arsenic affecting 70 million people. The disaster now underway may be the worst environmental catastrophe of the twentieth century.

Although the future of drug testing will be secure, it will be subject to radical changes with technological advances. Detection limits will continue to be pressed downward. Already, detection of a single atom, the ultimate quest for those seeking assays with greater and greater sensitivity has been achieved for some metals. Detectors for drugs with attomolar sensitivity have been described. Consequently, the race for sensitivity will continue until the ultimate limiting factor in the assay will be the background signal and probably not the limitation of the detector. As an interesting sidelight, if we could immediately jump ahead to the use of new drug assays that were one million-fold higher in sensitivity than our most sensitive assay we might be astounded. Almost certainly, we would surely find that our solvents, laboratory equipment and even our staff who are engaged in drug analysis would be contaminated with trace levels of drugs.

The miniaturization of assays will likely occur early in the next century. Assays-on-a-chip have already been constructed, but await successful development and marketing. Further technological advances in microfluidic devices will help complete the process.

Clearly, exciting changes already underway in drug testing technology will emerge in the next millennium that will amaze and astound even the most jaded analyst of today. Mankind's urge to question, experiment, and test the limits of the world in which we live is a characteristic that has brought us to where we are today and most certainly continue to propel drug testing along this same pathway.

WIELDING THE AWESOME POWER OF DRUG TESTING

Currently, over 20 million workplace drug tests are now being performed in the US by the government, military and private industry. A large portion of these tests is based on the DHHS guidelines for federal workplace testing although there is a considerable amount drug testing in private industry that does not fall under government purview. As of July 1, 1996, the same drug testing provisions have been applied to Canadian and Mexican rail and trucking companies that have US operations. Around the world, some workplace drug testing is performed in Europe, Australia, Asia and South America, but the US remains the primary purveyor of these programs.

The unique context of workplace drug testing should be distinguished from other forms of forensic drug testing. Workers are considered “subjects” rather than “patients”. The specimen tested generally is urine although hair testing is practiced to a small extent in the “unregulated” private industry. The test result is not for clinical diagnosis, but rather to support decisions regarding hiring, promotions, suspension, referral to employee assistance programs, accident investigations, and firing of employees. The specimen is most often collected under chain-of-custody conditions to prevent the possibility of tampering. The specimens usually are shipped to a commercial laboratory that is equipped to test hundreds to thousands of specimens per day. Initially, specimens are screened for illicit drugs by immunoassay. The use of automated analyzers allows these tests to be run rapidly and accurately in a highly sensitive mode. This first test by immunoassay eliminates the bulk of negative specimens from need of further testing. Presumptive positive specimens are then tested by GC/MS under carefully controlled conditions in which stringent quality control conditions must be met. All specimen results (including negative results) must be reviewed by a trained “certifying scientist” employed by the laboratory prior to release of results. Turn-around time for results from specimen receipt in the laboratory to time of reporting results averages 24–48 hours for most commercial laboratories. Results are sent to a Medical Review Officer (MRO) whose function is to evaluate the accuracy of the test results and determine if there are alternate legitimate medical explanations for positive results. Once the test results have been reviewed by the MRO, they are forwarded to the employer.

Generally, a positive workplace drug test requires definitive action on the part of the employer. If the test was conducted as part of a pre-employment screen, a positive test would generally preclude a potential employee from further considerations for employment. An existing employee who tests positive for illicit drugs might be subject to compulsory treatment, or punitive actions could be taken including loss of employment. A key consideration in workplace testing is the degree of accuracy of positive results. DHHS guidelines require that all positive screening tests be confirmed by GC/MS. Only specimens that contain the target drug or metabolite in concentrations equal to or greater than established administrative cutoff concentrations can be reported as “positive”. All other specimens must be reported as “negative” (unless there is evidence of attempted adulteration in which case they can be reported as adulterated or unsuitable for testing).

Urine is the primary specimen utilized in workplace testing programs, but there is increasing interest in alternate biological matrices for drug detection. Hair testing is currently practiced in the US on a small, but growing scale in the private sector, and there is additional interest in the possible use of saliva and sweat as alternate or complimentary matrices for drug testing. Even greater instrumental demands are required for drug testing with alternate biological specimens because of lower concentrations and differences in the biological matrix. Currently, confirmation testing by GC/MS is performed on urine specimens containing drug analytes in the low microgram/liter concentration range. The use of alternate biological matrices such as hair, saliva or sweat in workplace testing will require accurate drug measurements in the low picograms/liter concentration range.

Aside from workplace drug testing, forensic drug testing takes on a myriad of other forms in many countries including crime scene investigations, drugged driving, medical evaluations, insurance testing, drug prevalence testing, illicit drug seizures, rape investigations, sports testing, doping analysis and many other applications. In many cases, the analyst is challenged to discover and measure any one of literally thousands of psychoactive and toxic substances. Final identification (and measurement) is usually based on detection by two assays that utilize different separation principles, and by comparison to a reference standard (if available). The analyst's work may not be over once a toxin is identified, since accurate quantitation is usually needed. This requires an assay system that is specific for the analyte(s) of interest, provides a quantifiable response across a specified concentration range, and is reproducible. Results may be reported either in a qualitative or quantitative fashion; however, quantitative measures generally must be available even if only qualitative results are reported. The identification and measurement of toxic substance requires highly sensitive analytical methods and trained personnel to support such efforts. Frequently, certification of equipment and analysts is required.

Drug testing is frequently used in efforts to answer important toxicological questions. Was an illicit drug involved? Was drug taken intentionally? When and how much drug was taken? Could the psychoactive effects of the drug interfered with the individual's ability to perform? Was drug use an important factor in the outcome of an incident or crime? Many scenarios like these are addressed on a daily basis by forensic toxicologists in an attempt provide information. The process of drug testing invokes great power to those who are in the position of interpreting the test results. A positive drug result may have extremely important ramifications to individuals undergoing testing as well as others involved. Life, liberty, and monetary considerations may be decided based in large measure on toxicological results. The importance of such testing to individuals and society cannot be overstated, and consequently, so is the underlying technology that supports drug testing.