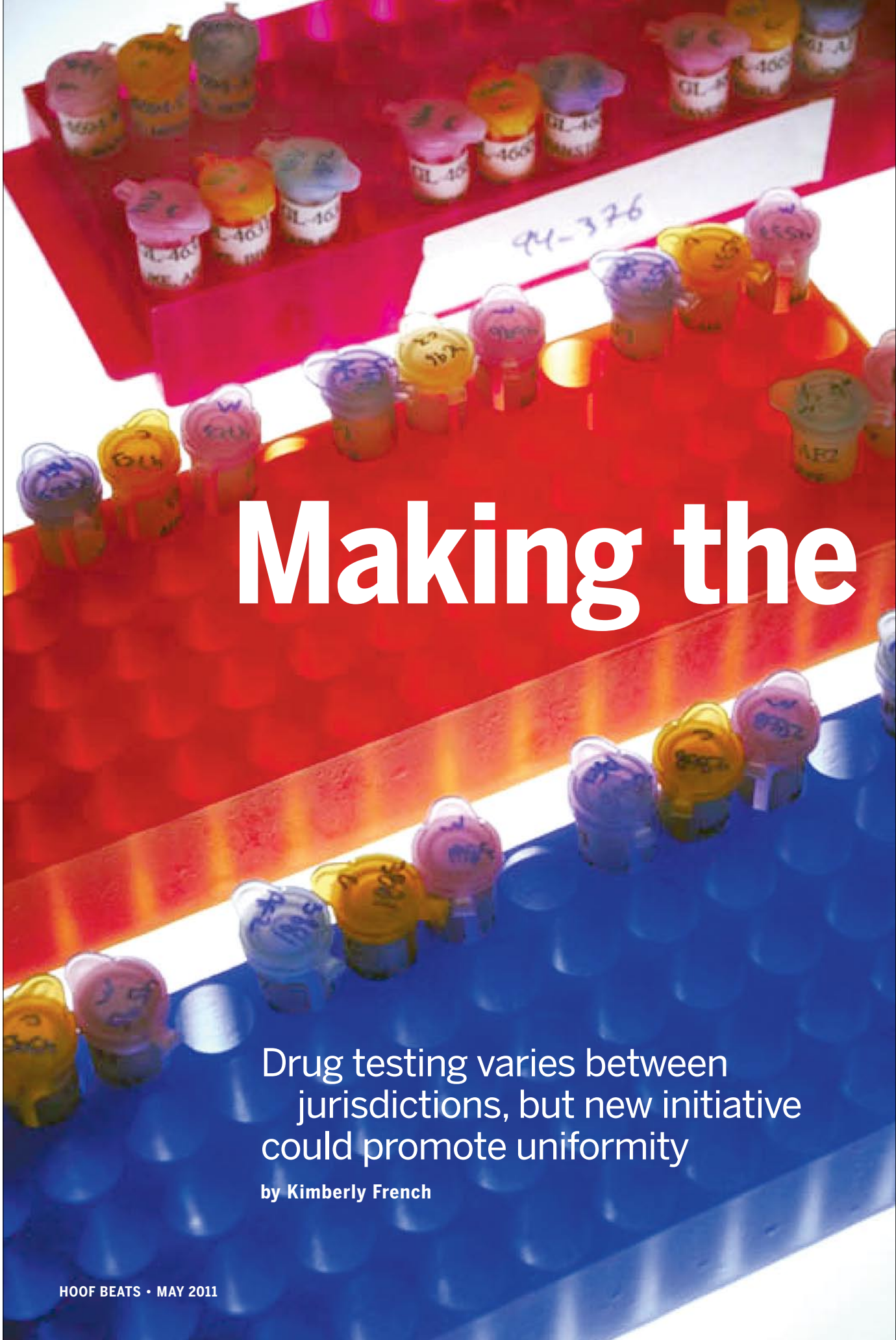

Making the

Drug testing varies between jurisdictions, but new initiative could promote uniformity

by Kimberly French

The conditions, both terrain and temperature, for the 1904 Olympic marathon contested in St. Louis could hardly be called idyllic. There was a series of eight hills for the 25.8-mile contest and by the time the race began, the thermometer had already crept to more than 90 degrees Fahrenheit.

In a testament to the brutal conditions, 18 of the 32 contestants failed to finish the race, but the man who ultimately was victorious enlisted some chemical aid to supply him with the necessary edge to best his colleagues.

Grade

By the 17th mile of the race, Thomas Hicks was walking. Somewhere around the 19-mile marker, he decided to take the advice of Hugh McGrath and Charles Lucas, who were members of his training team. He ingested 1/60 grain of sulphate of strychnine within a raw egg. Strychnine is poisonous, but it does stimulate the central nervous system in small doses.

After being passed by another runner who eventually was disqualified, Hicks was devastated, thinking he was already beaten, so McGrath and Lucas supplied him with another

IN THE LAB: John Merchant of the Analytical and Toxicology Laboratory of the Ohio Department of Agriculture works with a thermo LTQXL liquid chromatograph-mass spectrometer (LS-MS).

OPPOSITE PHOTO FROM USTA FILES • ABOVE PHOTO COURTESY OF THE OHIO DEPARTMENT OF AGRICULTURE

"There is very little incentive financially in this industry for a lab to become accredited," said Dr. Scot Waterman, **"and it is a difficult, time-consuming process, so the labs that have taken on this challenge deserve credit."**

dose of sulphate of strychnine with two more eggs and a glass of brandy. They also gave him a sponge bath on the course.

Hicks, who lost 10 pounds during the race, barely made it to the finish line. Not surprisingly, he collapsed after completing the race in 3 hours and 28 minutes, which is the longest finishing time in Olympic marathon history. After collecting his gold medal, he never competed in a marathon again.

Of course, people demanded he be stripped of his medal due to the use of chemical enhancements, but the Olympic Committee ruled in Hicks' favor as they had no established body of rules banning these substances from being used during competition. In fact, a system for drug testing was not implemented for Olympic athletes until the 1968 Games at Grenoble, France.

Horse racing has come under intense public scrutiny in recent years, since some of its participants use illegal substances to gain a competitive advantage, but it was the first sport to institute drug testing back in 1934, after coming under pressure from the FBI in 1933, with the introduction of the "spit box."

The Association of Official Racing Chemists was formed in 1947, more than 20 years before Olympic athletes were being tested, and since drug testing has been implemented in horse racing, it has been the most controlled, sensitive and specific of any sport in the world.

"Integrity is one of the things that drives the industry," said Dr. Cornelius Uboh, director of the Pennsylvania Equine Toxicology and Research Laboratory (PETRL), to HarnessWeek on Aug. 25, 2008. "To maintain that integrity, drug testing must stand at the forefront of whatever the (Pennsylvania Horse Racing) commission decides to do because that's where the commission can provide a level playing field to all participants."

Instruments and technology are essential to improving and maintaining the quality of drug testing; however, the entire process would never be possible

Types of Tests

Certified testing facilities use a wide range of testing procedures to ensure integrity within the sport. Here is a look at a few:

ELISA

During the 1980s, the Kentucky Horse Racing Commission issued a directive to an interdisciplinary team from the University of Kentucky to modify enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA) testing, for horse racing after realizing the sport may not have the necessary technology to test for new performance-enhancing drugs.

In ELISA, an undetermined amount of an antigen, which is a molecule that stimulates the production of antibodies, is applied to a surface, then a specific antibody is placed over the surface so the antibody can affix itself to the antigen. The antibody is connected to an enzyme and a substance is added so the enzyme can exhibit a signal, which is a usually a color change.

This idea of ELISA as an alternative to radioimmunoassay, which used radioactive antigens, was introduced in a paper in 1960 and was later formulated as a technique in 1971. It was the first test used for HIV because of its sensitivity and is often used in food-quality control, as well as other medical diagnostic scenarios.

ELISA TEST: The enzyme-linked immunosorbent assay, or ELISA, test is particularly sensitive to high-potency medications, can be conducted rapidly, and can be read by the eye.

"These ELISAs, when applied to routine testing, dramatically alter the withdrawal time necessary for clearance of therapeutic medications," wrote Dr. Scott Stanley and Dr. Cynthia Kollias-Baker in the *Review of Equine Drug Testing* in 1997. "A good example is acepromazine (the tranquilizer known as ace). When used at a therapeutic dose, acepromazine can be detected by thin-layer chromatography (TLC) for approximately 72 hours. The use of an ELISA for acepromazine, however, will allow detection for roughly 168 hours after administration."

HPLC

High-pressure liquid chromatography (HPLC) uses the basics of column chromatography where particles of a mixture are separated through a column by use of a solvent or substance that doesn't dissolve itself. Instead of the solvent dispersing through the effects of gravity, it is forced into the

PHOTOS BY ED KEYS

without the laboratories performing the testing and the funds from horsemen as well as the individual states to conduct the procedure. With no federal legislation in place to establish national guidelines, it is up to each of the country's 38 racing jurisdictions to implement their own individual testing policies and the labs in which they will be carried out.

Currently, 18 laboratories handle the entire volume of the drug testing for horse racing. Six of these labs, which include the Center for Toxicology Services, the University of Florida, PETRL, Industrial Laboratories, Truesdail Laboratories and the Ken Maddy Equine Analytical Lab, are accredited, while the Ohio State Racing Commission laboratory and the Iowa State University Racing Chemistry lab are in the process of becoming so.

"The accreditation standard for forensic labs is ISO 17025," explained Dr. Scot Waterman, executive director for the Racing Medication and Testing Consortium (RMTC). "There is very little incentive financially in this industry for a lab to become accredited and it is a difficult, time-consuming process, so the labs that have taken on this challenge deserve credit."

PREP WORK: An analyst pours a portion of each urine sample into test tubes. These sub-samples, called aliquots, are delivered to the thin-layer chromatography and ELISA testing areas.

ISO/IEC 17025 is the main standard used by testing and calibration laboratories. It was originally issued by the International Organization of Standardization in 1999. A second revised standard was introduced in 2005 and it includes the five elements of scope, normative references, terms and definitions, management requirements and technical requirements, but the two main requirements are management and technical. Management requirements apply to how the lab is operated and its quality, while technical requirements include how reliable and verifiable the laboratory's tests and calibrations are.

ISO 17025 is used by labs to instill a system that improves and consistently validates their test results and it is also

mixture at pressures of up to 400 atmospheres. This increases the speed of the process, allows the use of a much smaller particle size, provides a greater surface area for particle interactions and creates a much more minute separation of the mixture's components.

MS

In mass spectrometry (MS), a machine or scale called a spectrometer measures the weight of certain molecules and what they convert, too.

"The mass spectrometer provides specificity of identification and increased sensitivity over routine drug screening," wrote Stanley and Kollias-Baker. "Mass spectrometry operates on the principle that a charged particle moving through an electric field can be separated from other charged particles by producing charged molecules and molecule fragments and by measuring the mass of each. It works by the strategy 'divide and conquer,' chemically breaking down the molecules to smaller, more easily identifiable pieces."

GC-MS

In gas chromatography-mass spectrometry (GC-MS), a complex mixture is separated by a gas or vapor phase and the compound is entered in the mass spectrometer in that form.

"Mass spectrometry has become mandatory for many racing regulatory authorities and a requirement for laboratory accreditation," wrote Stanley and Kollias-Baker.

LC-MS

Liquid chromatography-mass spectrometry (LC-MS) combines the physical separation capabilities of both techniques to identify and detect chemicals in the company of other chemicals in a complex mixture. It is a very potent method and is used in many applications with very high selectivity and sensitivity, such as pharmaceutical studies and bioanalysis.

"The value of this technique has already been demonstrated with methods developed for steroids and butorphanol, compounds difficult to detect with other methods," wrote Stanley and Kollias-Baker.

MS-MS

In tandem mass spectrometry (MS-MS), a sample is introduced for ionization and subsequently these ions possess the characteristics of the individual drug compounds, which are called parent ions. These ions are further deconstructed into daughter ions and these provide a highly specific identification of the sought-after parent ion.

"The technology still has some drawbacks in that it is high-priced and requires the use of a highly skilled operator," wrote Stanley and Kollias-Baker.

In 2006, Dr. Cornelius Uboh and Dr. Lawrence Soma of Pennsylvania Equine Toxicology and Research Laboratory (PETRL) used this technique with liquid chromatography to produce a test for erythropoietin (EPO) and darbepoetin alfa. It was the first test of this kind for any sort of species, including humans, on the planet.

imperative to have accreditation from an accreditation body recognizing the lab's ability to perform its work.

Canada has two accreditation bodies, the Standards Council of Canada and the Canadian Association for Laboratory Accreditation, and most other countries have only one accreditation organization. The U.S., however, has six accreditation bodies.

The non-profit, government multidisciplinary groups are the ANSI-ASQ National Accreditation Board/ACLASS and A2LA. There is also the International Accreditation Service (IAS), Laboratory Accreditation Bureau (L-A-B), Perry Johnson Laboratories (PJLA), NVLAP and ASCLD.

"I think it's important to understand the process of how labs are selected and that is going to vary from state to state," said Ed Martin, president of the Association of Racing Commissioners International. "Some states have a statutory requirement that you have to use a particular lab in that state. There are other states where the labs are competitively bid out under multiyear contracts, and then there are some states where it's a matter of state policy and they aggressively urge their state representatives to do business with another state entity that has a lab."

"I think it's important to understand the process of how labs are selected and that is going to vary from state to state," said Ed Martin.

Accreditation does play a role in how states choose which lab they use, but many states such as New Jersey, Maryland and California are required by law to use a certain facility.

"The testing for California is done at the Kenneth Maddy Equine Analytical Lab (Maddy Lab) at the University of California at Davis (UCD)," said Dr. Rick Arthur, equine medical director for the California Horse Racing Board. "The lab is one of the best, if not the best, equipped laboratories for equine drug testing in the U.S. The testing is mandated to go to UCD by state law, but the lab is also one of the first ISO accredited equine testing labs in the U.S."

On March 1, 2010, the New York Senate and Assembly approved a transition in laboratory facilities from Cornell University to the State University at Morrisville. For nearly four decades, Cornell conducted all the testing and annually received more than 100,000 samples, but asked for a change in venue after requesting about $9 million in state funds to update their laboratory. According to New York law, the testing facility must be situated on a land-grant site.

Ohio tests its samples at the Analytical and Toxicology Laboratory located at the Ohio Department of Agriculture in Reynoldsburg. Accreditation, as well as several other factors, determined this lab's selection.

"While not a primary concern, location is a consideration," said Marty Evans, public information specialist for the Ohio

State Racing Commission. "As far as criteria for selection, that would be laboratory managers and staffing, availability of specific testing equipment, the ability of the laboratory to complete testing by the deadlines established, quality control measures, and proper accreditation."

Florida is another example of the state legislature designating a facility by law and then drawing up contracts with the laboratory.

"In 1998, the Legislature transferred the testing equipment and funding from the Department of Business and Regulation to the racing lab at the University of Florida, and the division currently contracts with the lab to perform these tests," said Sandi Copes, press secretary for the Department of Business and Professional Regulation. "We have maintained a successful partnership with the University of Florida because of the quality of service, the ability to handle our high number of annual samples and the convenience of an in-state facility, which keeps shipping costs low."

The Maine Health and Environmental Testing Laboratory conducts all testing for the state. Although they are not accredited through ISO 17025, they, like all the other labs not internationally employed, are members of the Association of Official Racing Chemists (AORC) and are reviewed annually.

"The lab is housed in the Department of Health and Human Services within the state government," said Henry Jackson, executive director of the Maine Harness Racing Commission. "We also use the state Federal Diagnostic Laboratory within the Maine Department of Agriculture for analyzing blood for total carbon dioxide gas levels and for our referee lab we use Iowa State University. We use an ELISA screening process and then GC-MS for confirmation."

The Iowa State Racing and Gaming Commission selected the Iowa State University Racing Chemistry Laboratory to complete its testing. The lab is accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and is also an active member of the AORC.

"While the AORC does not offer an 'accreditation' as such, we participate in the annual proficiency tests and I'm happy to say we pass that criteria," said Dr. Keith Soring, director of racing for the Iowa Racing Commission. "We are also in the majority through preparation for the ISO 17025 accreditation and should have that in place this year."

Since Minnesota does not have a state-funded lab, they contract the services out to the Industrial Labs in Colorado.

"Minnesota law requires a Request for Proposal (RFP) be sent out prior to a contract," said Dr. Lynn Hovda, chief commission veterinarian for the Minnesota

PHOTO FROM USTA FILES

Racing Commission. "Each potential laboratory is responsible for responding to the RFP and providing all the information required."

In 2009, the Governor's Blue Ribbon Task Force on the Future of Horse Racing in Kentucky advised the state to establish a state-of-the-art testing facility in the Bluegrass. Prior to that, the state had contracted out with such labs as the University of Florida, but on Dec. 14, 2010, HFL Sciences celebrated its grand opening in Lexington. The company hails from England.

"The laboratory selection process was lengthy and involved," explained Dr. Mary Scollay, equine medical director for the Kentucky Horse Racing Commission. "It was competitive, as more than one laboratory expressed interest in establishing a facility in Kentucky.

"The laboratory's quality of work, testing and research history and reputation were paramount. The analytic instruments need to provide state-of-the-art technology, having the ability to adapt as testing needs and methodology evolve. The selection process also acknowledged that drug testing is paid for by the host racetracks in Kentucky, so fiscal responsibility was another key criteria; the best testing for the best price."

Drug Testing Initiative

On Sept. 26, 2008, the Racing and Medication Testing Consortium announced it had adopted a five-part plan in response to the Jockey Club's Safety Committee meeting and to a plea from Alan Foreman, president of the Thoroughbred Horsemen's Associations, for a sweeping organization of drug testing at the Jockey Club's Round Table meeting that August.

This Drug Testing Initiative would be based upon the World Anti-Doping Agency (WADA) model code and would develop laboratory standards and accreditation criteria similar to ISO 17025 standards; expand current quality assurance and laboratory proficiency programs; develop a busi-

See **Grade** *on page 107*

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Grade *from page 71*

ness plan for the U.S. drug-testing infrastructure, including industry-sponsored research; and establish a post-doctoral and graduate student recruitment program for drug-testing research, plus review current sample collection strategies, such as the long-term storage of frozen samples.

"The Equine Quality Assurance program is the lynchpin of the accreditation program," said Waterman. "Basically, it is a combination of single-blind and double-blind samples that are submitted to the laboratory for analysis. The RMTC will work with respective racing commissions to have the results of the analysis independently analyzed for accuracy. There are penalties built in to the code for false positives or false negatives."

Waterman said WADA was chosen as a model for this program because it was very similar to what they wanted to accomplish.

"The biggest way our code differs is we do not have the ability to mandate it like WADA has with its laboratories," he said. "The state racing commissions do to some extent, so this will need to be a partnership between the RMTC and the commissions if this is going to work."

Martin, whose organization is a member of the RMTC and is recommending the Drug Testing Initiative for adoption to its members after minor modifications, agrees this concept has great potential for the entire racing industry.

"I understand a number of labs have voluntarily agreed to submit themselves for the accreditation effort for the RMTC and nobody has a problem with any of it," he said. "The main issue for the commissions is and has been whether the drug positives are overturned in a contested hearing. That is how the effectiveness of the labs is judged."

Kimberly French *is a freelance writer living in Kentucky. To comment on this article, e-mail us at readerforum@ustrotting.com.*

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