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Summary:

The Testing Integrity Program (TIP) is a collaborative partnership among analysts, veterinarians, and researchers with the goal of improving the quality of equine forensic science. TIP is committed to the development of validated analytical methods and collaborative quality assurance procedures.

The basis of the TIP approach is the validated method. A TIP validated method is one that has been developed by a primary TIP laboratory, repeated by a secondary laboratory, and reviewed by the TIP membership. Validation of each qualitative method involves experimental determination of the following method characteristics and specification of acceptance criteria, where applicable: Linearity, lower limit of detection, recovery, stability, specificity, ruggedness, and transferability.[1]

Collaborative quality assurance procedures include the creation and distribution to all TIP members of proficiency and administration samples to give each TIP members the opportunity to become fully proficient in the application of these methods. TIP also provides blind samples to interested authorities as part of the Quality Assurance services. TIP also functions as a Drug Administration Center (DAC) for the Association of Racing Commissioners International Drug Testing Standards and Practices Committee (RCI-DTS & PC).

Finally, TIP has a commitment to research to improve the quality of the forensic services TIP members offer. Research areas include the development of new analytical tests, development and validation of quantitative methods where these are required and working on the standardization of testing methods for dietary and environmental substances and ineffective traces of legitimate therapeutic medications.[2]

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[1]Validation of Qualitative Methods. TIP SOP document, personal communication, Dr. Richard Sams, Ohio State University.

[2]For the purposes of this communication, an ineffective trace is a concentration of a drug or drug metabolite in a plasma or urine sample that is unlikely to be associated with a pharmacological effect and that may therefore be considered pharmacologically ineffective.

Introduction:

In October 1995, a group of veterinarians, pharmacologists, analysts, administrators, academicians, and researchers met at the Keeneland Race Track in Lexington, Kentucky to discuss a new approach to Quality Assurance Program in the racing industry. Out of the October 1995 meeting came the Testing Integrity Program (TIP). TIP was organized to fill the void left by the demise of the previous Quality Assurance Program. The major difference is that this program is not driven by a single Drug Administration Center (DAC) and one laboratory director, but by multiple DAC's and group facilitators. The program is directed by its members who voluntarily joined TIP.[3] TIP maintains a collaborative partnership among analysts, veterinarians, and researchers with the goal of improving the quality of equine forensic science. TIP has a strong scientific basis in that many TIP laboratories are University based, and many members are career academicians. Currently TIP consists of members from The University of Kentucky, The University of California-Davis, Louisiana State University, Jamaica Racing Commission, The University of Pennsylvania, Industrial Laboratories Company, Oklahoma City Police Laboratory, University of Puerto Rico, Truesdail Laboratories, Texas Veterinary Medical Diagnostic Laboratory, The Ohio State University, The University of Florida, and West Chester University.

TIP is recognized by the Racing Commissioners International (RCI) as a provider of uniform drug testing and quality assurance program. RCI contracts with TIP to provide services that enhance, evaluate, and educate laboratories that serve the racing industry.

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[3] From the former TIP Website

(A) Method Development and Validation:

Once analytes for Proficiency Samples/Method Development Samples are selected by participating TIP members, and in accordance with the TIP/RCI contract, the process of method development is initiated.

The choice to develop a Standard Operating Procedure (SOP)/Method is voluntary. This choice is usually in accordance with knowledge or interest in a specific drug/medication. If participating TIP laboratories do not have first hand experience in a particular drug/medication, administration procedures are implemented for the process of method development and the production of a draft SOP. (Refer to Section (A) Sub-Section (III)for Administration Trials). When generating and preparing a SOP, no assumptions are made by the Method Developing Laboratory (MDL), and the proposed SOP is complete in its content and specifics as to allow for validation. The draft SOP is then sent to the Drug Administration Center (DAC) for enclosure with the samples when shipped to the participating TIP - participating Laboratories for method validation. In addition, a copy of the draft SOP is also sent to the TIP Information Officer. [Refer to Section (A) Sub-Section (X)]

(I) Analyte/Drug Standards: The analyte/drug standards are obtained commercially as 1mg/ml methanol stock solutions (Chromatographic Drug Standards, Alltech-Applied Science, State College, PA), or as authentic reference standards (Sigma Chemicals or specified pharmaceutical corporations) if chromatographic drug standards are not available. The chromatographic drug standards are prepared to provide the equivalent of the anhydrous free base or acid.

The DAC prepares 1mg (as free base or acid)/ml methanol stock solutions for those substances not available as chromatographic drug standards. The identity of these stock solutions

is verified by analysis of the native compound solutions and the TMS (trimethylsil) derivatives of each compound by gas chromatography/mass spectroscopy (GC/MS). The TMS derivative of each compound is prepared by evaporating 20µl of the stock solution and dissolving the residue in 20µl of ethyl acetate and 20µl N, O-bis-(trimethylsilyl) trifluoroacetamide catalyzed with 1% trimethylchlorosilane (BSTFA + 1% TMCS, Pierce, Rockford, IL), and incubated (Dry Bath Incubator, Dow Diagnostic, Model 100, Indianapolis, IN) at 70° C for 20 minutes. If, for any compound, the native or TMS derivative GC/MS results prove inappropriate to identify the compound, another derivatization method may be employed. An aliquot (1µl) of the solutions containing the native compound and of the TMS-derivative is then injected into a (GC/MS). The instrument employed is a Hewlett-Packard Model 6890 gas chromatograph (GC) equipped with a Model 5972A mass selective detector (MS). The column is an HP-5, 30 m x 250 m x 0.25 m. The carrier gas is helium with a flow rate of 1ml/minute. The volume injected is 1µl split-less mode at injector temperature of 250° C. Initial oven temperature is 70° C (held 2 minutes), ramping at 20°C/minute to 280°C (held 12 minutes). The total run time is 24.5 minutes. MSD temperature is 280°C. All 1mg/ml methanol stock solutions are stored at 0-5° C or at -20° C dependent on the stability requirements of the compound. Each participating laboratory is responsible for obtaining reference standards for the candidate analytes when the standards are available from commercial sources such as Alltech. In the event the standard is not commercially available an aliquot (0.5 to 1ml) of each stock solution and the mass spectral results will be provided by the DAC upon request.

II. Sample Generation and Preparation: Urine is collected from healthy and exercised Thoroughbred mares. The urine samples are pooled and verified to be blank by the TIP-MDL. This

verification step, however, does not apply to those samples which must be administration samples rather than supplemented samples. [Refer to Administration Trials, Section (A) Sub-Section (V through VII) for sample collection procedures]. Upon the confirmation of the urine sample being blank, it is thawed at 1^0 to 4^0 C one day prior to use. Once the blank urine sample is completely thawed, and just before use, it is allowed to warm to room temperature.

Proficiency/Method Development Sample Sets consist of five (5) samples, of which one (1) is a matrix blank. The samples are processed one at a time, working through the steps as quickly as possible to avoid excessive exposure of the standards to air. The procedure is as follows: The urine is measured into a 1300 ml portion using a glass graduated cylinder (Fisher Scientific, Pittsburgh, PA) and then poured into a two (2) liter glass erlenmeyer flask (Fisher Scientific, Pittsburgh, PA). The pH is then measured, (colorphast indicator, sticks , EM Reagents, Gibbstown, NJ) and recorded. The target analyte concentration is then calculated based on the volume of urine and supplementation at 20% above the target concentration. The supplemented urine sample is then mixed for fifteen (15) minutes by using a magnetic stirring plate (Therolyne, Cimarec 3). The 1300 ml supplemented portion is then decanted into thirteen (13) 100 ml aliquots in individual 125ml polyethylene sample containers (Nalgene, Nalge Company, a Subsidiary of Sybron Corp., Rochester, NY). Thirteen (13) aliquots are prepared for shipment to ten (10) TIP- participating laboratories, with one (1) aliquot for characterization, and two (2) as reserve samples to be used as split samples, if necessary. Twelve (12) of the sample containers are labeled (Avery Laser Labels, Diamond Bar, CA) and coded to indicate origin of samples, year of the set, the numbered sequence of the set for that year, the letter P or I (P signifies that the sample is a proficiency sample and has removed from the investigational

list; i.e. the limits of detection of the analyte and concentration have been previously analyzed by the participating members and a SOP for that particular substance has been validated . The letter "I" defines two situations. The first is that the sample is prepared at a concentration of an Investigational substance in which the capability to detect the analyte is uncertain. The second circumstance is that the sample is prepared with a proficiency substance in which the concentration is lower than the limit of detection. Finally the samples are coded with a letter (A through E), one specific letter for each individual substance used in the set. These letters signify to the DAC and the referee the identity of the sample, the content, and concentration. The thirteenth (13) sample container, a random sample, is labeled according to the analyte and the target concentration of that particular analyte, i.e. it is not coded. This sample is used for characterization as described in Section (A) Sub-Section (IX).

(III) Administration Trials: An administration trial is a vital procedure for method validation. Administrations are performed for three reasons. The first is due to DEA regulations for shipment of Scheduled Substances [Refer to Section (A) Sub-Section (V through VII)]. The second reason is interest in a specific drug/medication or metabolite. And the third reason is lack of knowledge about the metabolic pathways for a particular drug/medication. In these circumstances urine samples are collected and shipped to at least two (2) TIP labs, with chemical information and a literature search to give the analysts an overview of the chemistry and potential ways to detect the substance. From information gathered, TIP laboratories then collaborate to develop a "Draft SOP" for detecting that substance. When a "Draft SOP" is produced, the method is ready to be upgraded from investigational to proficiency for method validation by all TIP member laboratories.[4]

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[4] From the Former TIP Web Site

(IV) Horses: Five (5) Thoroughbred mares are used per Administration Trial. The horses used are approximately 450-600 kg, 2 to 10 years old, and are maintained on two 2 to 10 acres of pasture to permit a moderate degree of normal exercise. Each animal is given at least a two week wash-out period between trials. All administration studies are performed in accordance with the requirements and guidelines of the Animal Welfare Act. The horses are housed in isolated areas with strict access in a 12 x 12 stall 24 hours prior to treatment. All horses are sustained on grass hay and feed which is a 50:50 mixture of oats and alfalfa-based protein pellets, twice per day for the length of the administration trial which is seventy-two hours.

(V) Drugs/Medication and Administration: All drugs/medications, with the exception of DEA controlled substances, are commercially obtained from local pharmacies in human dose form, or from local distributors. The drug/medication is then administered to the horses in accordance to the appropriate dose and route of administration.

(VI) Sample Collection: Prior to administration, the perivulvar area of each horse is cleaned using cotton and Betadine Scrub. The first day of urine collection, (0, 1, 2, 4, 6, and 8 hours), is accomplished using Foley catheters that are inserted into the bladder with 24 oz. plastic Whirlpak bags attached to the end. Pre-administration (negative control) samples are collected fifteen (15) to thirty (30) minutes prior to administration. The remaining samples, (24, 48, and 72 hours) after administration, are collected using a Harris flush tube (24 FR x 60 in; Seamless, Ocala, FL). Urine samples collected at each time point are pooled, decanted into 100ml aliquots in 125 ml polyethylene sample containers (Nalgene, Nalge Company, a Subsidiary of Sybron Corp., Rochester, NY) and labeled according to drug, dose, route, date and horse number. The urine samples are then processed

as set forth by protocol in Section (A) Sub-Section (VIII) and (X) through (XII) where applicable.

(VII) Administration Sample Screening: If an administration trial is necessary, and before characterization, ELISA tests if available, are recommended to be performed on the samples collected from the horses following administration to verify that the target concentration(s) has been achieved, or to provide when appropriate, approximate concentrations of the drug in the administration trial samples.

(VIII) Sample Storage: Once the samples have been prepared, they are individually placed into 7 x 10, 2ml, light gauge poly bags (AABCO Plastics, Garfield Height, OH). They are then sealed by an electrical generated heat Impulse Sealer (Clamco Corporation, Cleveland, OH), sorted by analyte, one of each, making a set, for all participating laboratories. The sets are then placed in individual plastic storage bags and stored in an ultra cold freezer (Legaci (TM), REVCO Scientific, Model ULT 2586-7A, Asheville, NC) at -80^0 C until shipment.

(IX) Sample Characterization: After the sample preparation is complete, the random sample from each of the five sample sets is forwarded with all necessary information including a characterization reporting form to the MDL in order to establish that the sample contains the analyte, and / or that the analyte is present in the sample at the expected concentration. [Refer to Section (A) Sub-Section (XII) for shipping procedures]. The results of characterization analysis are reported to the DAC within five (5) working days. The proficiency sample sets are not forwarded to the participating laboratories until the MDL(s) have reported on the characterization sample(s) to the DAC.

(X) Flow of Information: Before shipment of any samples to the TIP Participating

Laboratories, the DAC sends all pertinent information concerning the shipment, including the draft SOP, to the Information Officer (IO). Once the information is reviewed by the IO, and at least five days prior to shipment, the information is forwarded to the TIP-Participating Laboratories in a TIP General Information Form disclosing the following: (1) For proficiency samples, indications of which samples are investigational and a reminder of the target concentrations for the candidate drugs, (2) if administration samples, indicated are the drug, dose, route, and approximate concentrations [Refer to Section (A) Sub-Section (VI)], (3) a specific laboratory code, and (4) the expected shipment and arrival dates.

(XI) Package Information: Information that is enclosed with the sample sets and sent to the TIP Participating Laboratories from the DAC is as follows: (1) For quality assurance verification, a cover letter consisting of the relevant information previously listed on the TIP General Information Form and instructions for the reporting of results, (2) a copy of the package insert for the analyte(s) if available, (3) a current draft of the SOP for all responsible/possible analytes, (4) a laboratory reporting form, and (5) two envelopes, one blank and the other pre-addressed for mailing to the referee laboratory. The completed reporting form with the individual laboratory code is placed in the blank envelope and is then placed in the addressed enveloped and sent to the contact person at the Referee Laboratory. The contact person then relays the reporting form to the referee. This procedure is followed to maintain confidentiality and anonymity among the TIP Laboratories.

(XII) Shipping Procedures: Each participating laboratory receives five (5) samples, one sample per analyte and/or concentration. Each sample set which had been stored in a plastic storage bag is placed in a 11" x 8.5" x 9" insulated Container (Model LB3, Tech Pak Inc., Peabody, MA),

packed in dry ice, and shipped priority overnight bonded commercial courier.

(XIII) Completion of a SOP: Once the proposed draft/SOP method has been verified by a minimum of two TIP-Participating Laboratories using the TIP SOP criteria for validation of qualitative methods, and acknowledged by the TIP referee, the method is then considered validated. The final version of the SOP is then prepared by the MDL for distribution to all TIP members and included with the verified methods list. Of this list, these drugs/medications then become candidates for proficiency sample sets.

(B) Quality Assurance Approaches:

An Internal TIP Quality Assurance mechanism is in place in that all drugs/medications that have been included to the verified methods list hence, subject to the scrutiny and validation of all TIP members, (Refer to Appendix I for procedural outline).

TIP also provides an external double blind sample program to assure the racing industry and the public that the testing laboratories can identify drugs and/or medications administered to horses and greyhounds to affect their performance in races. This program also assures that the chain of custody for racing samples from racetracks to testing laboratory does not compromise sample integrity.[5]

"Candidate drugs or medications for double blind sample testing are limited to those drugs and/or medications for which TIP has developed SOP's."[5] Once these have been established, the samples are either generated through administration, [Refer to Section (A) Sub-Section (III) through (VII) for administration procedures], or they are supplemented to the collected urine samples. The

[5] From the TIP/RCI Contract Agreement (March, 1998)

supplementation and sample custody procedures are as follows:

(I) Double Blind Sample Derivation: Urine samples are obtained from equine athletes through normal procedures established by each jurisdiction. They are then divided as a testing sample for the individual jurisdiction's contract laboratory and as a split sample in the event it becomes necessary to retest the sample. Once the testing sample has been cleared for illicit drug/medication, the split sample then becomes a candidate (by random selection) for a double blind sample.

(II) Chain of Custody: The random candidate samples are transported by a designated person from the testing barn/ "spit box" to the DAC. These samples are then supplemented, in accordance with the protocol of the contract agreement and by the approach set forth in the DAC supplementaion procedures [Refer to Section (a) Sub-Section (I)]. After the samples have been prepared, they are stored in an ultra cold freezer (Legaci (TM), REVCO Scientific, Model ULT 2586-7A, Asheville, NC) at -80^0 C until requested by the chief commission veterinarian. The samples are then transported by an appointed commission veterinarian to the shipping site and are distributed indistinguishable from the non-split/testing samples to the commission's contract laboratory for routine testing. (Refer to Appendix II for External Quality Assurance Procedural Flow Chart).

(C) Collaborative Research:

"When TIP members began collaborating, it was evident that there was a wealth of talent and resources at the table. The racing industry is well aware of drug testing questions requiring further explanation and scientific investigation, not to mention the continued vigilance to detect performance affecting drugs. TIP first detailed areas requiring research and felt the sharing and publication of research findings was essential. TIP then poled member laboratories and jurisdictions to outline

current research and resources available.[6] The end result of this initiative has produced an invaluable resource of information for the racing industry. "TIPs wealth of experts and knowledge are available for seminars, short courses and presentations to the racing industry.' Thus, 'TIP will aggressively promote educational opportunities and carry the message of integrity in testing to anyone interested.[7]

Research areas include the development of new analytical tests, development and validation of quantitative methods where these are required, and working on the standardization of testing methods for dietary and environmental substances and ineffective traces of legitimate therapeutic medications. As such, the results of TIP research are published in the refereed scientific literature, a major quality control mechanism in scientific research.

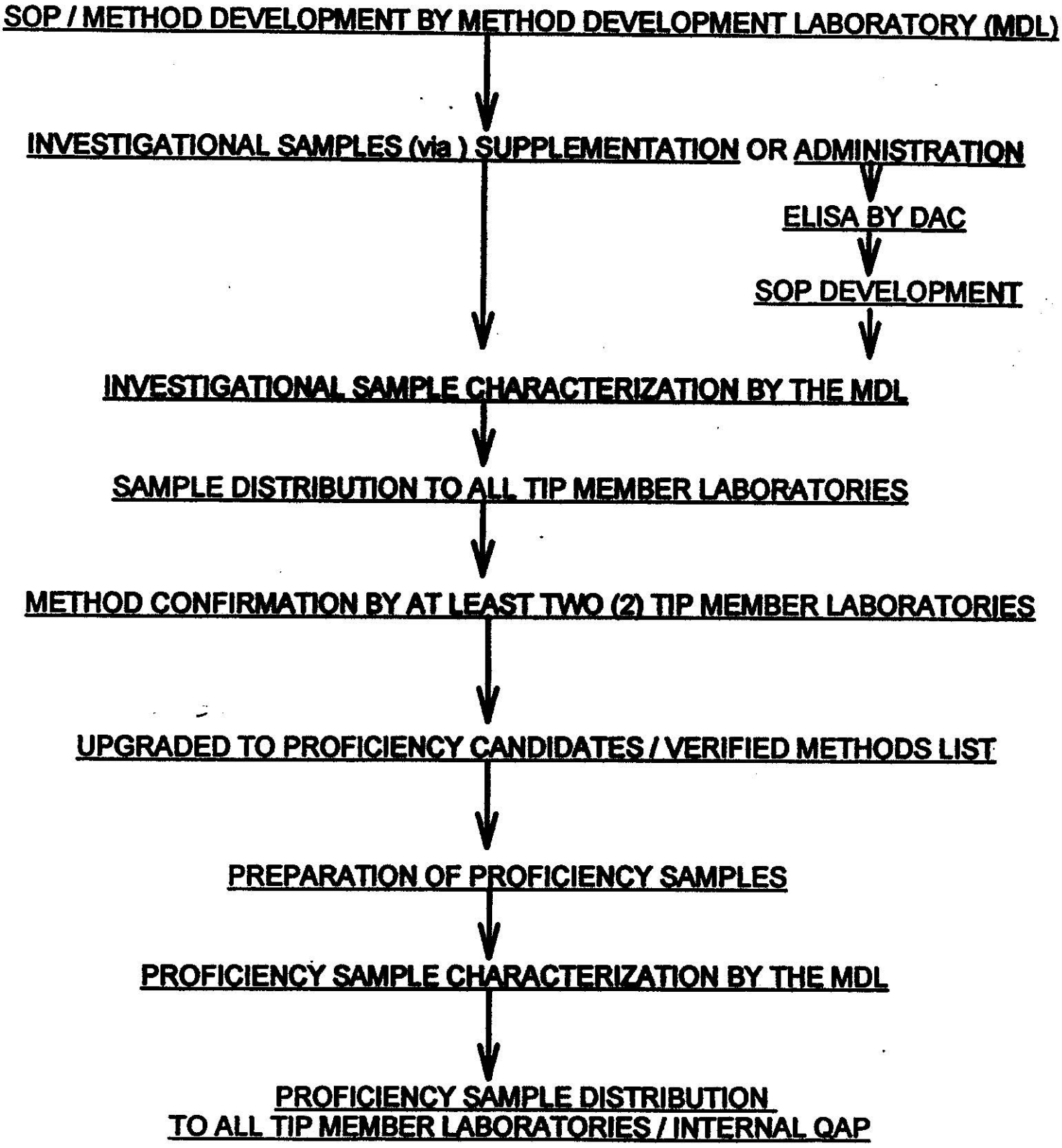
In conclusion, TIP's obligation can be summarized in three categories: 1) The validation of analytical methods of which an SOP is developed by a primary TIP laboratory, repeated by a secondary laboratory and reviewed by the TIP membership. 2) Collaborative Quality Assurance methods which include the creation and distribution of proficiency and administration samples to all TIP-Member Lasboratories to give each the opportunity to become fully proficient in the application of these methods in its daily screening of official samples. TIP also provides double blind samples to interested authorities as part of the Quality Assurance Services for the verification of testing approaches being used and to confirm that the integrity of the testing samples is not compromised. And the last 3), is TIP's commitment to research in order to improve the quality of the forensic services TIP members provide to the racing industry. This entails the sharing of knowledge and

[6] From the Former TIP Web Site

[7] From the Former TIP Web Site

expertise, the enhancement of educational opportunities, and the promotion of a just and fair establishment of testing procedures within the Racing Industry.

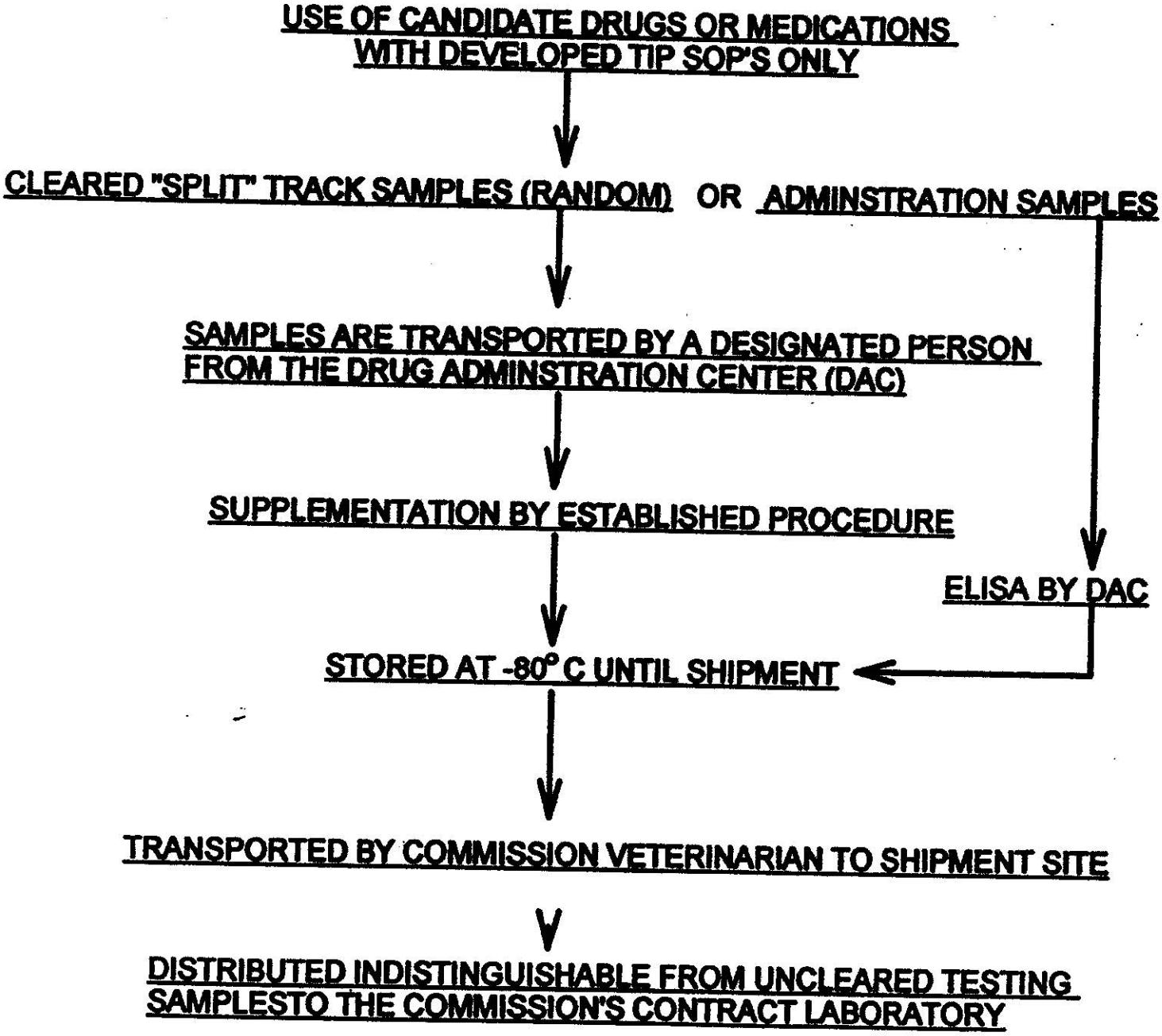
TIP METHOD DEVELOPMENT FLOW CHART



APPENDIX I

EXTERNAL QUALITY ASSURANCE PROCEDURAL FLOW CHART

GOAL: To assure the racing industry and the public that the testing laboratories can identify drugs and/or medications administered to horses and greyhounds to alter performance and that the chain of custody for samples from racetracks to laboratories does not compromise sample integrity.



APPENDIX II