



## **Test Barn Chain of Custody and Procedures: Considerations and Recommendations**

### **A Best Practices document by the Racing Medication & Testing Consortium and the National Thoroughbred Racing Association Safety & Integrity Alliance**

Pre and post-race sampling protocols at the racetrack test barn are the industry's front line for ensuring fair, safe racing through medication and substance integrity. Neither the RMTC nor the NTRA Safety & Integrity Alliance advocate a "one-size-fits-all" solution but rather encourage jurisdictions to adopt procedures relevant to their regulations and in consideration of any facility-specific needs. To that end the RMTC and the Alliance provide the following as Best Practice recommendations for the efficient design and operation of test barn facilities in coordination with effective chain of custody protocols. Deviation from these recommended protocols does not necessarily indicate an operating deficiency or a compromise in the chain of custody. The goal for accredited facilities is to demonstrate comprehensive, thoughtful and consistent test barn procedures that ensure safety of all participants, chain of custody integrity, and regulatory value.

#### **1. Identification of Horses for Sampling**

It is important that the stewards or judges, racing officials, regulatory veterinarians, horsemen, and test barn personnel know — in advance of the diversion of any horse to the test barn — what subset of the racing population is eligible to be subjected to testing. Conventionally, the stewards will select the winner of a race plus one or more "stewards'/judges' specials". Additionally, claimed horses and/or injured horses may be tested.

Consideration must be given to test barn capacity, sampling personnel, and estimated retention period for a tested horse when determining how many horses can be safely presented for sampling.

##### **A. From Unsaddling to Test Barn**

It is important to know whether local regulations specify the trainer, the association, or the regulatory authority as responsible for securing the horse from the time it finishes the race to the time it is presented at the test barn.

If the regulatory authority is responsible, it is ideal to have a representative of the authority or the racing association escort horses from the racetrack to the test barn. Alternatively, horses may be kept under visual surveillance by a designated authority representative. Note that the Trainer Responsibility rule is not waived when the regulatory authority does provide an escort.

It may not be practical for the regulatory authority to assign an escort to each horse, in which case the authority's designee must be alert to, and observant of, all horses identified for sampling. At a hearing, this individual may be required to provide testimony that no substances

were administered, nor was the horse in any other way ‘tampered with’ pending its arrival at the test barn.

#### **B. The Winner**

Testing notification may not be necessary for the winning horse; connections often assume the horse will be tested. That said, it may still be appropriate for a testing tag to be affixed to the horse’s bridle to identify the horse to observing officials and security personnel from the time it exits the Winner’s Circle and until it arrives at the test barn.

#### **C. The Stewards’/Judges’ Special**

A stewards’/judges’ special is a horse other than the winner that has been selected for post-race testing. Special selection considerations may include: beaten favorite; over-performers; runners out of high percentage shedrows; claimed horses; randomly selected runners; or horses associated with unusual wagering patterns.

It will be necessary to notify the horse’s connections, usually the groom and/or the trainer, that a selected horse must report immediately to the test barn for post-race sample collection. Most commonly, a test barn official, security official, or a designee of the stewards or the clerk of the scales receives this notice from the stewards and subsequently notifies the respective horse’s connections. It is helpful for exit gap attendants to also be notified in order to assist in diverting selected horses to the test barn.

A testing tag should be affixed to the bridle of any special selected for testing. These horses tend to exit the track as a group and a testing tag will clearly distinguish the horse after other identifiers (saddle towel, owner’s silks) have been removed.



#### **D. The Claimed Horse**

It is difficult to accurately project test barn facility and staffing needs when sampling is mandated for all claimed horses, and so advance consideration must be given to the scope of analysis required for samples derived from claimed horses.

Ideally, the samples derived from every horse presented for post-race testing will be subjected to the laboratory’s full scope of testing through the analysis of a paired sample. This may be an

unrealistic goal when the test barn becomes overcrowded with multiple claimed horses. Thus, blood-only sampling may be contemplated for some or all claimed horses.

Consult the drug testing laboratory in advance to verify that analysis of blood-only sampling affords a scope of analysis that includes;

- all substances regulated by serum/plasma threshold, and
- substances having an extended post-administration detection interval (*i.e.*, anabolic steroids) which, as a consequence, could impact post-race testing results in the horse's subsequent starts.

For blood-only samples, the laboratory may require additional sample volume. Any such requirements should be conveyed to test barn personnel in advance.

#### **E. The Injured Horse**

Considering the medical needs of the injured horse it may be necessary to collect samples on the racetrack and under conditions where the trainer (or his designee) is not present as a witness. Prior to sample collection from any injured horse chain of custody must receive careful consideration and a protocol established.

It is prudent to seek advice from the authority's legal counsel with respect to:

- The individual(s) that may serve as witnesses to a collection in the absence of the trainer or his designee;
- How the sample should be managed prior to its transfer to the test barn given that the sampling veterinarian's focus may be, most appropriately, on managing the injured horse.

For the injured horse, it may be practical to divert the horse ambulance to the test barn before returning the horse to its barn. Blood sampling can be performed on the ambulance, and the sample immediately taken to the sample processing area of the test barn. Urine collection under these conditions may be unrealistic.

**Note:** Immediately post-exercise the relative fluid volume of the horse's blood is reduced. Comparatively less serum or plasma can be obtained from a tube of blood collected immediately post-exercise when compared to that obtained from standard post-race testing. For this reason, when horses are sampled immediately post exercise, additional tubes of blood should be collected to ensure an adequate volume of serum or plasma for analysis. If these samples are to be used for enforcement purposes it is important to acquire sufficient volume for both a primary and split sample.

If sampling is performed after emergency medications are administered, it is important to notify the testing laboratory of administered drugs so that analytical results can be reconciled with the administered emergency medications. The administration of typical emergency medications will not interfere with modern testing methodologies for other substances.

## F. The Deceased Horse

Provisions must be made to secure the body from the time of death until its transfer to the veterinary diagnostic laboratory for necropsy.

It is recommended the logistics of regulatory sample collection at necropsy be discussed, in advance, by the regulatory authority (including legal counsel) and personnel from the drug testing and veterinary diagnostic laboratories.

As above with the non-fatally injured horse, it is recommended to collect additional tubes of blood.

Urine samples may be collected at necropsy. It is not uncommon for urine to drain as the deceased horse is loaded into the ambulance. It may be helpful to stock single-use urinary catheters on the ambulance, or supplies to facilitate free-catch collection before, or during, loading. Even if chain of custody requirement cannot be satisfied, urinalysis may provide useful intelligence for the mortality review process.

## G. Veterinary Emergencies

The regulatory authority has an obligation to monitor the health of those horses and to promptly respond to emergent medical conditions. Horses within the test barn should be observed by regulatory veterinarians for signs of injury, epistaxis, unsoundness, or distress. Some conditions, such as minor lacerations, and run-down sores may be addressed on site and with the horse still detained for completion of sampling.

Other conditions, such as onset of lameness, may require blood-only sampling and release of the horse to the care of the trainer and private attending veterinarian. Horses so released should be placed on the Veterinarians' List and designated ineligible to enter until released by a racing regulatory veterinarian.

In the absence of a diagnosis, it may be appropriate to transport a lame horse back to its home barn via equine ambulance to reduce risk of further injury.

Some conditions, such as 'heat stroke' or 'heat exhaustion', require immediate veterinary intervention and can preempt sampling. Protocols must be in place to rapidly address these conditions. This can be achieved by summoning a private practicing veterinarian to initiate treatment. Alternatively, the regulatory authority's veterinarian can maintain a limited inventory of emergency medications for use in treating affected horses.

**Note:** In most cases, the treated horse can be sampled after the administration of emergency medications. The laboratory must be notified of the medication(s) administered so that the detection of those substances will not initiate confirmatory analysis and the issuance of a Report of Finding.

The stewards/judges should be notified whenever the sampling of a horse is abbreviated (blood-only or excused entirely from sampling) at the discretion of the official veterinarian.

## 2. Which Samples to Collect

Budgets constraints may encourage cost-saving measures in the test barn. Often the suggested solution is to collect blood-only samples. This practice is not advisable. Collecting blood-only samples risks inadequate or incomplete testing, as follows:

- Laboratory screening methodology for some substances is limited to urine – if urine is not submitted, those substances will go undetected;
- Some substances are quickly eliminated from the blood and can only be effectively regulated through analysis of urine; and
- Regulatory thresholds for some therapeutic medications require detection in urine or in both urine and blood – dual thresholds ensure that the medication was not administered at a therapeutic dose in proximity to racing and that a sub-therapeutic dose was not administered on race day.

**A paired (blood and urine) sample is necessary for the laboratory to apply its full scope of analysis and provide the regulatory authority with comprehensive analytical support to its medication regulations.**

There is diminished value in blood-only sampling of numerous horses. The better option would be to acquire paired samples on all horses but test a fewer number. This would ensure that tested horses are competing within the regulations.

## 3. Within the Test Barn Enclosure

### A. Chain of Custody

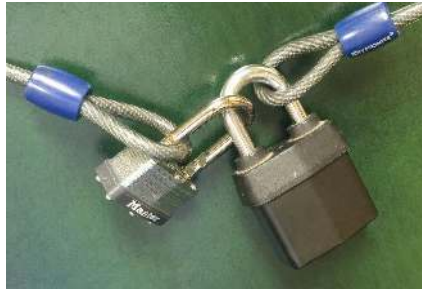
Chain of custody are the procedures to account for the integrity of each sample by tracking its handling and storage from the time of collection to the final disposition of the specimen. At base, the chain of custody demands that a piece of evidence must be secured without any chance of tampering, loss, or contamination, if it is to be admissible in court. Currently, and in response to increased laboratory proficiencies, defense attorney arguments are more likely to challenge chain of custody rather than the analytical process itself. Maintenance, and documentation, of chain of custody cannot be overemphasized.

### B. General Considerations

The test barn enclosure is a restricted area. Access should be limited to current license-holders and on an as-needed-only basis. Usually a groom, and perhaps a second individual, will attend to the horse through cooling out and sample collection. The trainer of the horse may also attend, but more often the groom will be that trainer's designee.

The facility should be secured at all times, and locked when not in use by regulatory personnel.

To accommodate off-hour/off-season access by emergency responders it may be advisable to install double intertwined locks on external fences or doors. A key to either the regulatory authority's lock or the racing association's lock can then afford access the facility.



Official samples retained within the enclosure should be maintained in locked refrigerators or freezers and be accessible only to the regulatory authority.

The regulatory authority is responsible for oversight of all activities within the enclosure.

It is recommended that bathing supplies (*i.e.*, shampoo, sponges, buckets, sweat scrapers) be provided in the test barn. This practice eliminates the introduction of materials into the enclosure that may contain regulated or banned substances with the ability to impact the results of drug testing. When infectious disease (*i.e.*, Influenza, Herpesvirus, or Strangles) is diagnosed or suspected on the premises, it may be appropriate to permit a trainer to provide his own bathing supplies. These materials should be inspected before being allowed into the test barn enclosure. Unlabeled products should not be permitted.

It is also recommended that materials brought into the test barn by the horse's attendants be inspected. Halters, lead shanks, bandage cutters, scissors, and stable sheets or coolers all have legitimate use and may be permitted within the enclosure.

Medicated shampoos, ointments, skin braces, other topical products, or orally administered products should not be permitted within the enclosure.

Food products, beverages, smoking and use of nicotine products should be strictly prohibited within the enclosure and these prohibitions should be clearly indicated by bilingual signage prominently displayed at the enclosure entrance.

That said, it may be necessary to establish a break area within the restricted area for test barn employees where food and beverages can be stored and consumed. In that case, the break area should be separate and distinct from the areas where horses are handled, sampling supplies are stored, and samples are processed/stored.

### **C. Arrival/Departure Procedures**

1. Upon arrival at the test barn, each horse's identity must be verified by 1) inspection of its lip tattoo or freeze-brand, 2) microchip scan, or 3) physical description on its registration papers.

Each horse's respective attendant should flip the horse's lip to display its tattoo to the test barn staff. To avoid the potential for physical transfer of pathogens to the hands or clothing

of test barn personnel or other horses, test barn staff should avoid contact with a horse's mouth and nostrils.

If test barn personnel are required to contact the horse's nose or mouth, single use disposable exam gloves should be worn and changed before contact with another horse. If unexpected contact occurs, the individual should promptly wash his/her hands with soap-- before contact with sampling materials or another horse.

2. Station a security officer at the single access point of the enclosure. This officer will inspect and record racing licenses or track-issued credentials for all individuals accessing the enclosure. Individuals lacking current credentials and official business within the test barn should be denied access.

The security officer records on a daily log the time each horse enters the enclosure, and the names and pertinent licensing information of those attending with the horse.

If, for a given horse, no attendant has the necessary credentials, test barn personnel should detain and visually monitor the horse within the vicinity of the test barn and contact the stewards/judges for guidance. If this is not practical, it may be necessary to allow the horse and unlicensed attendant into the enclosure. However, it is not advisable to allow an unlicensed individual to serve as an official witness to a collection. Prompt communication with the stewards/judges is key to ensuring that sample collection is adequately documented when these events occur.

3. When sampling is completed test barn personnel will authorize the horse's departure.

Test barn personnel may attach a "release tag" to the horse's halter when sampling is completed. The security officer is then instructed to permit only tagged horses to exit. As the horse exits, the security officer retrieves the tag for disinfecting and reuse. This procedure will prevent horses from exiting the test barn before all sampling is completed.

The security officer will record the time of departure in the daily log. This establishes a record of when, and how long, the horse was in the test barn and what other horses and personnel were present during that interval.

4. Once a horse exits the secure enclosure, unless accompanied by a test barn employee, it cannot return. The chain of custody has now been interrupted and cannot be restored.
5. After the last sampled horse has left the test barn, the security officer submits the daily log to the test barn administrator. The log will be retained as evidence for the event of a Report of Finding.

State records retention requirements should be consulted for guidance on disposal of records associated with cleared or negative samples.

#### D. Sample ID card

Under no circumstances, at any time during the analytical process, should the laboratory know, or be able to determine, the identity of the horse or trainer associated with a specific sample. The “blinding” of samples is a fundamental aspect of testing integrity.

Upon notification from the stewards/judges of the horses to be sampled from a given race, the test barn administrator completes a Sample ID for each horse. The Sample ID card is the official document that establishes a link between a horse and a sample number.

It is critical that the card be completed correctly and legibly. Errors in recording gender or medication status (e.g., Salix) can result in testing delays; the issuance of erroneous Reports of Finding; or the inability to pursue a medication violation and may delay distribution of purses. The Sample ID card also is a record of who collected samples from the horse, and who witnessed the sampling on behalf of the trainer.

Date: AUG 24 2017  
Track: EP 0017002  
Salix  Adjunct   
HORSE: Le Chevalier  
TATTOO NUMBER: M03458  
COLOR: Gr/fo SEX: G AGE: 3  
RACE: 9 FINISH: 1st  
TRAINER: T. Proctor  
SAMPLED BY: [Signature]  
SAMPLED BY: [Signature] BLOOD  
WITNESS NAME: [Signature]  
WITNESS LICENSE: 36894  
COMMENTS:

At the conclusion of each race day’s sampling activities, a designated test barn employee should review and verify the contents of the Sample ID cards by reconciling each with the horses’ information in the official racing program. For example, accurate gender reporting is necessary for the laboratory to support the authority’s anabolic steroid regulations.

The Sample ID cards are then sealed in an envelope and retained in the test barn or transferred to the designated officials. The information on the cards remains confidential from the time sampling is completed until the issuance of a report by the official laboratory, thus insuring that:

- testing is conducted impartially;
- samples associated with individual horses or trainers cannot be singled out for enhanced or reduced testing, and;
- no one—either at the racetrack or the laboratory—is able to make a connection between a sample and a specific horse until all testing has been completed.



**E. Test Barn Day Log**

It is important that a record exists documenting any and every individual involved in collection, processing, and shipping of each specific sample. The Sample ID card and the Day log in combination should constitute a record of everyone contacting a given sample within the test barn. This is a key component of chain of custody, and the effort required to establish this record is well justified at an administrative hearing.

Recording information on this form (example below) establishes a composite record of all test barn activities for each race date.

**Official Test Barn Report**  
 Date: JULY 21, 2014  
 Test Barn Supervisor: M.C. SCOLLAY, DVM (PRINT)  
6 TH DAY OF RACING AT BAL

SAMPLE NUMBER	RACE	FRESH	BUCKET NUMBER	HORSE	GENDER	TRAINER	Urine	Blood	UA
0077767	1	1	1	FRODOH N. IRISH	G	W. BEAVIS	✓	✓	✓
0077768	1	3	2	BECKY'S SURGE PGT (CLAIMED P. MAREQUIS)	G	R. VOGLWER	✓	✓	✓
0077769	2	1	3	ZEN TAN TARE	H	M. VAJEZ	0	✓	✓
0077770	2	6	4	LEGS GALORE	H	R. COMBEST	✓	✓	0
0077771	3	1	5	HAI'S IMAGE	G	R. ROSE	✓	✓	✓
0077772	3	2	6	ARIPKA SUGAR	G	M. MACHARG	✓	✓	✓
0077773	4	1	7	TATER PATCH	F	J. WILBY	0	✓	✓
0077774	4	2	8	ROSSES KING VANTY	F	K. CRUMPLER	✓	✓	✓
0077775	5	1	9	SAM ANDIEN'S TURE	R	R. IRWIN	✓	✓	✓
0077776	5	4	10	SHICKERS MOUSE	G	J. WARD	✓	✓	✓

COMMENTS: 7770 - HEAT STROKE IN TEST BARN.  
 Txd w/ DETOMIDINE, EXCUSED BLOOD-ONLY

INJURED HORSES: 0 WORK HORSES: 2

This horse raced without Salix. Laboratory must be notified in order to respond to presence or absence of furosemide in this horse's post-race sample.

Instruction to sample horse from the right jugular based on Salix administering veterinarian's notice of treating left jugular.

A urine sample was not collected from this horse. An explanation is provided in the Comments line. Lab must be notified that horse was treated with detomidine before blood sampling was performed.

**4. Sample Collection**

**A. General Considerations**

1. The regulatory authority should request from its official laboratory documentation of its requirements for sample collection, processing, and storage prior to collection of samples. The information requested could include:
  - a. What volume of urine/blood is necessary for proper testing;
  - b. Whether the samples require centrifugation. If so consider items such as:

- i. what is the minimum and maximum interval after collection for starting the centrifuge;
    - ii. the speed and duration of centrifugation;
  - c. How should samples be stored in the test barn (*e.g.*, refrigerator versus freezer, etc.);
  - d. How should samples be packed for shipment to the laboratory?
2. The regulatory authority's duty to protect the integrity of collected samples begins well before any horse is presented at the test barn. The inventory of yet-to-be-used sample collection materials should be stored in a secure location accessible only to test barn personnel. These materials must be protected from willful tampering as well as incidental exposure to regulated substances.
3. The importance of a paired (blood and urine) sample cannot be overstated. The scope of analysis that can be applied to a paired sample is far greater than what can be applied to blood or urine alone. As noted above, in the face of constrained operating budgets, the regulatory authority is better served by testing fewer, paired samples.
4. The timing of sample collection can affect testing results, particularly with respect to threshold substances having short half-lives (*e.g.*, flunixin [Banamine®]).

With a half-life of less than 90 minutes (a 50% decrease in blood concentration every 90 minutes), the concentration of flunixin in a sample collected 15 minutes post-arrival will be substantially higher than the concentration detected in a sample collected from the same horse 45-60 minutes post-arrival.

In order to establish fair enforcement practices, it is advisable to schedule blood collections at a consistent amount of time post-race for all horses (*e.g.*, 30 minutes after the horse's arrival at the test barn) and independent of urine collection, which is less time-sensitive.

5. Inappropriate timing of blood sample collection can also impede the laboratory's analytical capabilities due to inadequate sample volume.

During exercise, within the horse's blood, the ratio of cellular material to fluid increases dramatically. A blood sample collected immediately post-exercise may yield only 2-3mls of serum. If sampled 30 minutes post exercise, the serum yield may be 5-6 mL as a normal fluid balance has been re-established within the body.

It is for this reason that additional blood tubes must be collected when sampling is performed immediately post-exercise (*e.g.*, when sampling the injured horse on track).

It has been observed that in some test barns it is protocol that horses in the last race undergo blood collection immediately upon arrival at the test barn, enabling the phlebotomist to depart shortly thereafter. If all other horses from every race are not consistently sampled immediately upon arrival (which is not advisable), this practice establishes regulatory prejudice against participants in the last race.

6. When third-party race day furosemide (Salix®) is implemented it is both possible and advisable to define separate administration and sampling sites.

A standard policy of furosemide administration, for example through the left-side jugular vein and sampling from the right-side vein, eliminates the risk of confounding testing results—which can be the consequence of inserting a collection needle through a subcutaneous deposit of furosemide left at the time of administration.

If a trainer requests administration to the right side, the treating veterinarian can notify the test barn of the deviation, and post-race sampling (if required) can be performed on the left side.

7. Sample collection may be expedited, and made safer, by maintaining a readily-consulted log of individual horses having sampling idiosyncrasies (*e.g.*, “right jugular not patent, always sample left side”, “kicks”, “strikes”, “bites”, etc.).

## **B. Blood Collection**

1. Blood should be collected directly into sealed vacuum tubes.
  - a. Alcohol swabs may be used to assist visualization of the jugular vein and to remove debris from the collection site. This practice can be particularly helpful in the winter months when sampling horses with long hair coats. However, the application of an alcohol swab may make the apprehensive horse more so, and thus its use is at the discretion of the phlebotomist.
  - b. The use of a needle and syringe with subsequent manual transfer to tubes is discouraged as it represents an opportunity for loss of sample integrity.
  - c. For horses identified as having allergies to silicone, it may be necessary to collect blood using a needle and syringe if silicone-free blood collection needles are not readily available.
  - d. When a syringe is required for blood collection, the phlebotomist should wear single-use exam gloves until the blood has been transferred to the collection tube and a tamper-evident seal is in place over the rubber stopper.
2. The trainer’s representative must be afforded the opportunity to inspect the bar coded number labels applied to the blood collection tubes and verify that each is consistent with the unique number on the horse’s Sample ID card.
3. While in the presence of the trainer’s representative, the phlebotomist will cover each tube’s rubber stopper with tamper-evident tape.

The tape should not be dislodged or manipulated for any reason until the samples arrive at the laboratory. Damage to, or disruption of, the tamper-evident seal should disqualify the sample from further analysis as chain-of-custody cannot be defended.

4. Blood samples should never be opened at the test barn. The efficiency in centrifugation of serum separator or plasma separator tubes to separate the fluid component from the cellular component of the blood eliminates any justification for this.
5. The volume of blood required by the official laboratory should be established before any sampling is performed. Likewise, the laboratory should indicate the appropriate volume to be collected for the 'split' or 'B' sample.
6. The split sample is a separate and distinct sample, collected at the same time as the 'primary' or 'A' sample and retained for the sole purpose of analysis at an approved referee laboratory upon incidence of a Report of Finding.

The 'split' sample is not the portion of the 'primary' sample that remains after the official laboratory has completed its analysis.

The absence of a split sample for analysis by a referee laboratory can be grounds for dismissal of a medication violation.

7. Regardless of necessary sample volume dictated by the laboratory, a minimum of two tubes should be submitted per each test subject.
8. The type of tube used—glass/plastic; serum separator/plasma separator/heparinized/EDTA—depends on the matrix to be analyzed (serum or plasma), and the regulatory authority's intentions with respect to long-term sample retention.

Glass tubes are not suitable for freezing. If samples are intended for long term, frozen storage and glass tubes are used for collection, the serum/plasma must be decanted into plastic storage vials. This practice establishes vulnerability in the chain of custody, and, if done in a test barn, is only recommended if subsequent analysis is intended to be for investigative or research purposes, only. However, if the regulatory authority intends to be able to pursue a finding detected in the analysis of a retained frozen sample, the trainer's representative must be afforded that opportunity to observe the decanting, labeling, and sealing process.

9. The selection of the proper collection tube type and volume is made in the regulatory authority's consultation with the official laboratory and in consideration of local regulations or statutes governing drug-testing procedures.

### **C. Urine Collection**

1. Urine collection is achieved via free-catch as the horse voids. Unlike blood collection, it does not utilize a closed, or sealed system and additional care must be exercised to ensure the integrity of the resultant sample.

It is advisable that sterile urine collection cups be provided to the regulatory authority with snap-on lids in place and anchored with adhesive seals or provided in sealed, individual

packaging to demonstrate that the interior of the cup has not been exposed to human contact or environmental detritus, or compromised in any other way.

If the lid is missing or has been dislodged, or the individual packaging damaged, the collection cup must not be used.

2. The urine collector should don a pair of disposable exam gloves before inserting the lidded cup into the collection stick, a long handled device with a receptacle for the urine collection cup at one end. The gloves will be worn until the sample has been poured off into two containers ('A' and 'B' samples) and the two containers sealed.

The lid remains on the cup until the horse is brought into the stall for urine collection. If the lid is to be replaced on the cup after sampling (*see*, Section C-6, *infra*) the regulatory authority should ensure that it is stored in a manner as to limit the exposure to potential contaminants.

If, at any time after the lid has been removed, and/or the urine collector's gloved hand, or anything other than the collected sample contacts the inside of the urine cup, the cup and its contents must be discarded and replaced with a new cup.

3. Urine collectors should not cover the open container with the palm of their hand as can occur in the event a horse is fractious and jostles the collector or the collection apparatus. The glove is not sterile, and it should not even be considered 'clean' as it was used to open the stall door, contact the horse, and may bear dirt, dust, horse hair or sweat – that could be transferred to the sample.



4. The typical collection cup can receive an amount far in excess of the sample volume required for analysis. The jurisdiction's laboratory should be consulted to determine what constitutes an adequate sample volume for testing. While the sample cup need not be filled in order to declare urine collection a 'success,' it is important to remember that both the 'A' and 'B' samples must originate from the same collection.
5. Until employees have a clear understanding of the acceptable minimum sample volume, it may be helpful to mark the outside of the urine cup with a 'fill to' line. It is preferable that the sample be acquired from a single void. However, if the horse produces a small amount of urine, less than the required minimum, that sample cup should be covered and sealed pending the subsequent collection into another cup of a sufficient volume to meet the

laboratory's requirement. The combining of these specimens should be witnessed by the trainer or his/her representative.

6. After a sufficient amount of urine has been caught in the collection cup, the collector may place the properly stored lid back on the cup and will then deliver the sample to the processing area.
7. The trainer's representative may remain in the stall during urine collection or observe from the outside through a porthole in the stall door.
8. The regulatory authority determines how long horses will remain in the test barn for the purpose of urine collection. This period generally ranges from 60 minutes to 2 hours. However, the regulatory authority can, at its discretion, increase the retention period for an individual horse. If at the end of the retention period, a horse fails to provide an adequate urine sample, that horse's postrace sample is designated a 'blood only' sample.
9. The urine collection stick should be thoroughly rinsed between uses.

#### **D. Collection of Other Matrices (*i.e.*, hair)**

In general, if the sample collection is achieved through a non-closed system (*i.e.*, pulling mane hairs, swabbing saliva), gloves should be worn by the collector throughout the process. If not, substances on the collector's hands or secreted through the pores can be transferred to the matrix and confound, or invalidate, testing results.

### **5. Sample Processing**

#### **A. General Considerations**

The sample processing area should be further access-restricted to regulatory authority personnel actively engaged in processing samples and the trainers' representatives witnessing their samples' processing.

Trainers' representatives should have clear visibility on the processing of their horses' samples, but should not have physical contact with the samples. All samples should be sealed with tamper-evident tape and have applied a unique identifier (*e.g.*, bar-code or sample number).

The trainer's representative, after witnessing the sealing of samples, should sign the sample card. In addition, the representative's license number should be recorded in the event that they need to be identified at a later date.

#### **B. Blood**

1. If the blood matrix to be analyzed is serum, the whole blood must clot before the serum can be separated from the cellular material.

The sealed blood tubes should be allowed to sit upright at room temperature for 30-45 minutes and then centrifuged.

Following centrifugation each tube should be inspected to ascertain if complete separation of serum from the red blood cells has been achieved. If necessary, centrifugation should be repeated one time.

Following centrifugation, the blood tubes should be refrigerated pending packing and shipping to the laboratory. Once centrifuged, it is no longer necessary for the tubes to be stored in an upright position.

2. If the blood matrix to be analyzed is plasma, anticoagulants are present in the collection tube and the blood should therefore not clot. If the blood clots, or forms clumps, the collection should be considered failed and the horse re-sampled.

Some plasma collection tubes contain a separator gel that will establish a barrier between the plasma and the cellular material after centrifugation; others do not. The official laboratory should provide clear instructions for the processing of plasma samples within the test barn.

#### **C. Urine**

1. After a sufficient amount of urine has been caught in the collection cup, it is promptly transferred to the sample processing area.
2. Two urine sample containers are placed on an absorbent towel or pad, and the urine is poured directly from the collection cup into the sample containers.

It is critical that the urine be poured into the sample cups. The introduction of syringes or other devices into a sample risks the transfer of urine from one horse's sample to another horse's.

Once the caps are firmly affixed, the exterior of each sample cup should be wiped dry with a disposable towel.

3. The urine collector is then able to remove and dispose of the exam gloves.
4. Tamper evident seals should be applied over the urine containers, the corresponding Sample ID bar code sticker applied, and unless otherwise instructed by the laboratory, the samples should be placed in a secured, locking freezer pending shipment to the laboratory.

#### **D. Other Matrices (*i.e.*, hair)**

For other matrices, sample collection processes should be clearly established, in consultation with the official testing laboratory, and prior to any attempts at sample collection.

1. For hair sample collection, gloves should be worn, and changed between horses. The gloves help prevent substances on the collector's skin or secreted through the pores from adhering to the sample and thereby confounding or invalidating test results.
2. Pulling combs or scissors should be washed, rinsed, disinfected, and dried after each use. When not in use, these tools should be secured in a clean, dry location.

## 6. Sample Management

### A. General Considerations

At the conclusion of the day's sample collections one individual should be tasked with:

1. Verifying the information recorded on the Sample ID cards and the documents to be transferred to the laboratory,
2. Affirming that all Sample ID cards have been signed by the phlebotomist, urine collector, and the trainer's representative,
3. Ensuring that the individual samples collected from the day are accurately reflected on any documents submitted to the laboratory for that day (*e.g.*, sample inventory forms) and that all such samples are included in any other chain of custody documents, and
4. Affirming that for every primary sample there is a corresponding split sample.

### B. Primary Samples

The official laboratory will provide instructions for packing the primary samples for transport.

If samples are shipped via commercial carrier (*e.g.*, FedEx or UPS) it is recommended that the regulatory authority enable automatic delivery/delay notifications for each shipment's tracking number.

### C. Split Samples

1. The split sample is, as noted above, a separate and distinct sample, collected at the time of the 'primary' or 'A' sample and retained for the sole purpose of analysis at an approved referee laboratory upon incidence of a Report of Finding.
  - a. It is not the portion of the 'primary' sample remaining after the official laboratory completes its analysis.
  - b. The absence of a split sample for analysis by a referee laboratory may be grounds for dismissal of a medication violation finding.
2. "RMTC Guidelines for Split Sample Analysis" can be found at: <http://rmtcnet.com/wp-content/uploads/2016/03/Split-Sample-Guidelines.pdf>. This document explains the obligations and expectations of both the regulatory authority and the referee laboratory that performs split sample analysis.
3. The RMTC's "Guide to the Split Sample Analysis Solicitation Form" can be found at: <http://rmtcnet.com/wp-content/uploads/2016/03/Guide-to-Split-Sample-Analysis-Solicitation-Form.pdf>.

This document explains each section of the Request for Split Sample Analysis form, and clarifies the importance of the information provided to potential laboratories. The goal of the solicitation is to ensure that laboratories capable of performing the requested analysis



respond to the request. Providing incomplete, or inaccurate, information to candidate laboratories can jeopardize the regulatory authority's ability to prosecute a rule violation.

4. The interactive PDF form "Request for Split Sample Analysis" referenced in the Solicitation Guide above can be downloaded at: <http://rmtcnet.com/wp-content/uploads/2016/03/Request-for-Split-Sample-Analysis-Form-4.pdf>
5. It is recommended that Split Samples be retained by the regulatory authority rather than transferring them automatically to the official laboratory.

Retention can be on-site in the test barn if sufficient, secured storage capacity exists. Alternatively, an off-site location may be utilized. If an off-site storage location is utilized the transfer must be documented to maintain chain of custody.

Whatever facility is used, the regulatory authority must ensure that the samples are secure, storage conditions are consistent, there is an alert or notification system in the event of a power outage, and that samples are accessible to authorized personnel should a split sample analysis be required.

6. Split samples should be retained, at a minimum, until the regulatory authority receives final testing clearance from the official laboratory.

It is not advisable to pre-determine an 'automatic' disposal date. Should the laboratory request a deadline extension for completion of its work, split samples will need to be retained until the laboratory issues its report.

Retained split samples corresponding to passed/cleared samples may have investigative or research value which warrants a longer retention period.

7. Split samples (urine and blood) corresponding to a primary sample for which a Report of Finding has been issued should be retained indefinitely, pending disposal authorization by the regulatory authority's legal counsel. While a finding may be reported in a specific matrix (*i.e.*, serum), administrative hearing officers often accede to trainers' requests for analysis of the corresponding urine sample.
8. The process for split sample retrieval and shipment to an approved reference laboratory may be specified in the regulatory authority's regulations. If it is not, a split sample protocol should be established and applied consistently in all cases. The important aspects of the split sample process are:
  - a. Verifying the security of the split sample from the time of collection and entry into storage until it is retrieved for analysis.  
A log should be maintained that records all activity associated with the split sample storage unit: when, by whom, and for what reason has the storage unit been accessed.
  - b. Verifying that the sample ID number is consistent with the sample ID associated with the laboratory's report of finding.
  - c. Inspecting the sample container to verify that the tamper-evident seal is intact.

- d. Ensuring that correct information accompanies the split sample to the reference laboratory.
- e. Packing, shipping, and tracking the split sample for arrival at the referee laboratory in the best possible condition.
  - i. Determine if the split sample laboratory is capable of receiving shipments on the weekend. If not, it is advisable not to ship after Wednesday of any given week to provide reasonable assurance that the sample will be received no later than Friday.
  - ii. A frozen sample thawed, or a chilled sample at room temperature, may undergo degradation that can impact the results of the analysis.
  - iii. Monitor the shipment for in-transit delay or failed delivery.

## 7. TCO<sub>2</sub> Testing

Many jurisdictions perform total carbon dioxide (TCO<sub>2</sub>) testing on all participants in one or more races as a part of race-day sampling. It is important to note that these samples have different considerations than post-race testing with regard to sample timing and split samples. Sample processing will be similar to that of post-race test processing.

### A. Sample Collection Timing

1. Pre-race blood sampling for TCO<sub>2</sub> is preferred. Pre-race sampling should be collected 45 minutes (+/- 15min) pre-race and approximately three hours after furosemide (Lasix, Salix) administration.
  - a. Furosemide produces an elevation in TCO<sub>2</sub> averaging approximately 1.7mmol/l for a standard 250mg IV dose administered four (4) hours prerace. The furosemide effect has been considered in the standard 37mmol/l regulatory TCO<sub>2</sub> threshold. Because of this, timing of the TCO<sub>2</sub> sample is crucial to ensure an accurate reading.
  - b. Sampled runners should remain in a secure detention area until race time.
2. Post-race sampling of horses for TCO<sub>2</sub> testing should be discouraged. But, if necessary:
  - a. The selected horses must remain in the test barn for a minimum of one and one-half (1.5) hours prior to sampling to permit TCO<sub>2</sub> to approach actual values.
  - b. The subject horse must be reasonably well cooled off prior to sampling. Post-exercise hyperventilation can cause artificially low TCO<sub>2</sub> findings.

### B. Sample Processing

Samples must be handled in a consistent manner and must not be frozen.

Blood samples must be processed and tested using standardized, reproducible, validated procedures designed to preserve chain of custody and process consistency.

In general, the sooner samples intended for TCO<sub>2</sub> testing are analyzed post collection, the better, in order to minimize sample degradation that results in decreased TCO<sub>2</sub> values. This typically requires that any split sample travel immediately to the reference testing laboratory.

Later verification by an independent reference laboratory — as would be done for a finding in a post-race sample for a controlled therapeutic medication or banned substance -- is not feasible.

## **8. Out of Competition Sampling**

Out of Competition samples represent another aspect of test barn protocol. Most often, these are blood-only samples but can include hair samples and other biologic matrices. The handling of samples largely depends upon where the sample is collected and for whom.

For any sample collected, it is important that the following guidelines are adhered to:

- a. The individual collecting the sample should be a veterinarian licensed in the jurisdiction in which the collection occurs, or a veterinary technician as permitted by state law; and carry appropriate credentials to display to the trainer.
- b. The individual collecting the sample should inform the trainer or assistant trainer of the purpose of their visit – to collect out of competition samples. In the trainer’s absence, available barn personnel should be notified and instructed to inform the trainer, assistant trainer, or barn foreman;
- c. The individual collecting the samples should identify the horse via microchip, tattoo number, freeze-brand or markings;
- d. The individual collecting the sample should ensure that they have an appropriate location to safely sample the horse;
- e. Adequate sample should be collected to allow for a split sample (*i.e.*, 3 blood tubes);
- f. The trainer’s representative should witness the labeling of sample collection materials, the collection of the sample, and the sealing of the sample; and
- g. The trainer’s representative should sign the collection card provided to acknowledge they witnessed the collection and sealing of the sample.

### **A. On Association Grounds**

If a sample is collected by the regulatory authority having jurisdiction over the association grounds’ location, the sample – once collected and sealed – should be promptly returned to the test barn. Thereafter the sample should be managed consistent with test barn handling of post-race samples.

If the sample is collected on association grounds but requires transport to another location for sample processing, the sample should remain on ice packs/dry ice in a cooler or refrigerated until it can be processed. Chain of custody maintenance is particularly important when sample collection is performed at a location remote from the sample processing and shipping site.

The split sample should be retained pending the Commission’s authorization for disposal.

### **B. In-state, Off-site**

If a sample is collected within the testing authority’s jurisdiction, but not on association grounds, the sample, once collected and sealed, should be brought to a location where the sample can be managed as a post-race sample. Pending transfer and processing, samples should be kept on ice

packs/dry ice in a cooler or refrigerated, and in the custody and control of the individual performing the sampling.

The primary sample can be sent to the laboratory and the split sample retained in a secured manner with post-race samples.

### **C. Out of State**

Where a jurisdiction (the Requesting Jurisdiction) asks another (the Sampling Jurisdiction) to obtain an out of competition sample, the Requesting Jurisdiction should include the following information with the request:

- a. Instructions regarding the Requesting Jurisdiction's procedures for sample collection and processing;
- b. Horse information (name, tattoo/microchip number/description, likely location of horse – *e.g.*, last work location);
- c. Contact information for someone at the Requesting Jurisdiction (including weekends/evenings);
- d. A copy of the relevant Out of Competition sampling rule and/or a letter from the Requesting Jurisdiction indicating its authority and potential penalties for refusal;
- e. Sampling materials (*e.g.*, blood tubes, needles, needle holders, urine specimen cups);
- f. Sample identification card, and associated documentation;
- g. identification labels for individual blood tubes/urine containers;
- h. Sufficient evidence tape/materials to seal samples;
- i. Cold packs for shipment;
- j. Packing material for shipment; and
- k. Pre-made/pre-paid courier labels for shipping.

The Sampling Jurisdiction should then follow the above instructions based upon whether the horse is located on Association Grounds in the Sampling Jurisdiction (section 8.A.), or elsewhere in the Sampling Jurisdiction (section 8.B.).

If the horse cannot be located within the respective jurisdiction, the Sampling Jurisdiction should inform the Requesting Jurisdiction as soon as practical.

## **9. Facilities**

### **A. Design, Layout**

1. Secured Perimeter, single access point:

The test barn should have a secure perimeter with a chain link or solid fence fully surrounding the enclosure (see picture below). This prevents individuals from leaving or entering the test barn without approval of the regulatory authority. This also prevents the general public from interacting with the horses.

The entrance should be staffed at all times that there are horses in the test barn by either a regulatory authority or association employee.



2. Signage:

Install signage (*i.e.*, English and Spanish) at the entrance to the test barn indicating that:

- a. The test barn is a secured, monitored area;
- b. Only licensed personnel are allowed to enter;
- c. There are limitations on the number of individuals that can accompany a horse in the test barn; and
- d. Food, drink, and nicotine products are prohibited.



3. Stalls should:

- a. Be of sufficient size for horse to turn around comfortably,
- b. Be constructed of easily disinfected materials such as fiberglass or sealed wood. Unsealed wood can harbor bacteria and pathogens and is difficult to effectively clean.
- c. Include a tie ring at the corner or center of interior wall,
- d. Have dutch doors with latches that allow release from the inside and outside, and include an observation portal (see photo).



- e. Consider a corner wall panel for test barn personnel to stand behind or as an escape avenue from especially fractious horses (see photo).



9. Lighting:

The test barn should have adequate lighting in the stalls for safe collection of urine and venipuncture. Stall lighting should be installed sufficiently high or caged to protect the horse from injury.

10. Ventilation:

The stalls should have adequate air flow in all seasons including access to windows, regardless of season, and fans for warmer months.

11. Bathing Area:

Wash stalls should be available for the trainers' use. They should be located in an area that is line-of-sight visible to test barn personnel and located within the fenced enclosure.

The ideal bathing stall is a 3-wall standing stall with a concrete floor, rubber mats, and drainage (see photo). Test barn staff should rinse bathing stalls and remove debris between horses for cleanliness.

Bathing equipment (*e.g.*, sponges, scrapers, and buckets) may be provided but should be kept clean and disinfected.



#### 12. Equipment:

Water buckets should be provided by the regulatory authority. Each horse should be assigned its own bucket for the period of time it remains in the test barn. Buckets may be re-used through the race day but should be thoroughly washed and disinfected between uses.

**Note:** After the prescribed contact time for the disinfectant (see subsection *D.4. General* for recommended disinfectants), generously rinse buckets with fresh, potable water before filling them for horse use.

Trainer supplied equipment, if allowed, should not be shared between horses.

#### 13. Break Room:

The employee break room should be isolated and located away from the location of sampling and sample storage. Signs should be posted reminding employees that all food and beverages must be consumed in those areas and may not be brought into areas of sampling collection, handling, or storage.

### B. Surveillance

#### 1. Lines of Sight:

It is important to be able to observe horses in the test barn enclosure at all times. Ideally, the test barn has an open design that allows viewing of horses at all times when they are

not in a stall (see photo). If that is not possible, based on the test barn layout, observers can be staged to maintain visibility on horses walking or being bathed.



## 2. Security Cameras:

Security cameras are recommended throughout the test barn facilities. Key points for camera locations include stalls, shed rows, entry, laboratory, sample processing, and storage areas.

Beyond direct monitoring of horses during their time in the test barn, recording of these areas will enable the racing authority to submit tapes into evidence when test barn practices/specific instances are challenged. Recordings should be maintained until respective tests are cleared by the official testing laboratory.

Each regulatory authority should consult its attorney regarding the limitations/disclosures required for video and/or audio recordings.

## C. Signage

Adequate signage should be installed at the test barn entrance to inform licensees of test barn regulations. Bilingual signage should be used if available.





#### D. Sanitation

1. When the facility is in use, manure deposited in walking areas and wash racks should be removed as promptly as is reasonably possible.

2. Bedding:

Maintain adequate bedding in each stall. The decision to use sawdust, straw, or shavings should be made in consideration of bedding conventionally used by horsemen at that location. If multiple bedding types are in use, it is advisable to have one or more stalls bedded with each type to afford horses reasonably familiar environments in which to sufficiently relax to produce a urine sample. For shavings, however, use only bagged pine shavings to avoid potential contaminants originating from other trees. To decrease the amount of dust, it is advisable to lightly dampen shavings prior to the start of races and as necessary through the day.

Between horses, wet or soiled bedding should be removed.

Stalls should be completely stripped at the end of the race day and any wet spots in/on the flooring should be addressed. The stalls should remain empty overnight to allow any remaining wet areas to dry. All used bedding should be completely removed from the test barn enclosure.

3. Hazardous waste removal:

Install adequate facilities for hazardous waste removal including a sharps bucket and provisions for disposing of biological samples in accordance with all applicable regulations.

4. General:

It is recommended that all stall walls and bathing areas are washed and disinfected weekly. All visible organic materials (*i.e.*, urine, manure, bedding, dirt) should be removed from stall walls and bathing areas prior to washing with a nozzled hose and detergent. Pressure washing is not advisable as it aerosolizes organic matter and bacteria or viruses contained within. These organisms can be subsequently activated and infect horses.

Following the label instructions, sanitize the cleaned areas with a solution adequate to disinfect in the presence of organic material. Recommended disinfectants contain oxidizing agents, phenols, or quaternary ammonium compounds. A useful resource for selecting an appropriate disinfectant can be found at:

<http://www.cfsph.iastate.edu/Disinfection/Assets/CharacteristicsSelectedDisinfectants.pdf>

**Note:** Dilute bleach solution is ineffective and should not be used.

If constructed of amenable materials, walking areas and flooring should also be washed with detergent and disinfected on a weekly basis, as above.

## E. Sample Processing Area

The sample processing area should be maintained in a clean and orderly manner. This should include layering disposable towels or other materials to prevent contact between spilled sample and the workbench.

Access to this area should be further restricted to test barn employees and designated trainers' representatives who are witnessing sample processing.

The witness should be separated from the sample processing area, such as by a work counter, but be allowed to observe the process. Do not share writing pens or other devices across the counter. Provide separate writing pens for test barn employees and witnessing representatives. You have little knowledge of, or control over, what substances (legal or otherwise) may be present in or on the individuals who present horses for sampling. It is advisable to constrain the ability of those individuals to inadvertently introduce substances into the environment where samples are collected or processed.



When serum is the regulated matrix, it is advisable that samples be centrifuged on-site to separate the serum from the cellular components within the sealed collection tubes. This can also apply to plasma. If a centrifuge is necessary but not supplied by the laboratory, one should be purchased that meets specifications provided by the laboratory.

A landline and television for monitoring races are also important. The entire area should have a door that locks from the outside allowing it to be secured when test barn personnel are not present.

When samples are stored at the test barn, it should have a backup power source and/or an automatic notification system for when power is interrupted. This is necessary to protect the integrity of the samples as repeated thawing and freezing can degrade the sample.

Alternatively, there should be a protocol whereby the racing association will notify the regulatory authority of any power outage affecting the test barn that occurs outside of normal

working hours. If a backup power source is unavailable, it is recommended that the regulatory authority have a plan for transportation of samples under chain of custody to an alternate location to maintain sample integrity.

## 10. Personnel

### A. General Considerations

It is important to remember that the work of the test barn staff includes the handling of evidence that may be introduced in a hearing. Because of this, professionalism should be stressed to each employee. Regulatory authorities may want to consider a uniform or, at a minimum, a dress code for test barn staff. This could include:

- a. Polo shirts, vests, or jackets identifying test barn or regulatory staff;
- b. Jeans, khakis, or walking length shorts;
- c. Minimal jewelry; and
- d. Footwear appropriate to working with horses.



### B. Attending Veterinarian

A licensed veterinarian should be at the test barn, at all times when horses are present, to monitor their health and welfare and assist in any emergencies. The test barn veterinarian should observe the horses to ensure they are recovering well from the races and capable of remaining in the test barn for urine sampling.

It is acceptable to maintain a limited stock of emergency medications in the test barn. These medications should be isolated and locked in an area away from locations of sample collection, processing, and storage. It is also helpful to have spare equipment—halters, lead shanks, bandage cutters, and shoe pullers—to make available for those who arrive unprepared or in the event their equipment breaks.

### **C. Veterinary Technicians**

In authorized jurisdictions, veterinary technicians are allowed to perform venipuncture for post-race and other sampling. These veterinary technicians must meet the requirements of supervision of the veterinary board. Other individuals, including veterinary assistants, are not licensed to draw blood regardless of supervision.

### **D. Urine Collection**

Appropriately trained lay-staff are capable of collecting urine samples. It is important that they are trained regarding safety around horses, appropriate sample handling, and chain of custody prior to sampling horses.

### **E. Integrity and Confidentiality**

Integrity is vital. Test barn employees should not be in the grandstand during the race day. Employees should remain at the Test barn at all times except when assigned to tag/escort horses on-track, when on a scheduled break, or otherwise instructed or authorized by the test barn veterinarian. Test barn staff should limit time on the backside and in the grandstand outside of work duties.

All regulatory authority employees must follow ethical regulations regarding wagering on horses – under no circumstance should an employee bet on horses that are racing during their shift. Potential conflicts of interest should be disclosed at the time of hiring, and subsequent disclosures should be made if conflicts arise over time.

Potential conflicts of interest can include the following:

- Being related to another licensee;
- Personal relationship with another licensee;
- Racehorse ownership;
- Business relationship with another licensee (*e.g.*, farrier, private veterinarian, trainer, tack shop); and
- Employment by another licensee whether at the track or off association grounds.