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Part A

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PREAMBLE

The 2009 version of the document *Accreditation requirements and operating criteria for horseracing laboratories* was revised in 2015 and approved for publication by ILAC in 2016.

PURPOSE

The purpose of this document is to provide:

- **Part A**: A compilation of test-method-related requirements for horseracing laboratories that accreditation bodies have submitted.
- **Part B**: Recommendations for establishing the presence of prohibited substances that have been agreed within the horseracing industry.
- **Part C**: Additional recommendations on compliance with an appropriate performance specification, and the adoption of harmonised definitions for terms commonly used by racing chemists.

RECOMMENDATIONS TO ACCREDITATION BODIES

Accreditation bodies are encouraged to submit additions or other modifications to Part A through:

ILAC Secretariat
E-mail: ilac@nata.com.au

Additions may, for example, be compliances or non-compliances that assessors have noted.

Suggestions on Parts B and C would be welcome and should be sent to either:

The President of the Association of Official Racing Chemists
E-mail and other contact details can be found on the AORC website:
http://www.aorc-online.org

or

Dr Terence Wan,
Convenor of the ILAC-G7 Revision Working Group
E-mail: terence.sm.wan@hkjc.org.hk

With reference to compliance with an appropriate performance specification, accreditation bodies are encouraged to identify the performance specification met by a horseracing laboratory in its scope of accreditation. Some examples for the scope of accreditation are:

<table>
<thead>
<tr>
<th>Testing field</th>
<th>Chemical testing <em>or</em> Forensic testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials tested (test object)</td>
<td>Equine and canine body fluids <em>or</em> Body fluids, tissue and excreta from animals; materials that an animal may have received or may have been intended to receive</td>
</tr>
</tbody>
</table>
### Tests performed
Qualitative and, where relevant, quantitative analyses for prohibited substances as defined by the International Federation of Horseracing Authorities, or . . . as defined by the Rules of Racing of such-and-such racing authorities or regulatory bodies.

### Techniques used
In-house methods XXX to YYY or such-and-such analytical techniques or both.

### Recommended Additional Information
Meets the performance specification of the International Federation of Horseracing Authorities or (within the United States) . . . of the Association of Racing Commissioners International or (within Canada) . . . of the Canadian Pari-Mutuel Agency or . . . of such-and-such racing authority or regulatory body.

1. A flexible scope of accreditation is generally required in doping control testing, and this can be formulated in accordance with ILAC-G18: 04/2010, Guideline for the formulation of scopes of accreditation for laboratories.

2. There can be circumstances where a laboratory requires a flexible, technology-based scope of accreditation. Accreditation bodies shall refer to relevant parts of ILAC-G18: 04/2010 in assessing a laboratory for a technology-based scope of accreditation, which would not include a list of test methods.

### AUTHORSHIP
This document was first put together in 1994 and revised in 1996 by an ILAC working group convened by Dr David L. Crone. This is the second revision since that time and was undertaken by a working group within the ILAC Accreditation Committee (AIC) convened by Dr Terence S. M. Wan. **Part A** (accreditation requirements) was compiled and revised by the AIC working group. **Parts B and C** (operating criteria) were prepared and revised by the horseracing industry in conjunction with the working group.
INTRODUCTION

The general requirements for accreditation of laboratories are laid down in ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*. These requirements apply to all types of calibration and objective testing but need amplification in certain cases.

**Part A** provides interpretation of some of the requirements of ISO/IEC 17025 for horseracing laboratories and **Parts B and C** detail operating criteria that should normally be adopted.

Where there are differences of interpretation, ISO/IEC 17025 is the authoritative document, and individual accreditation bodies will make a judgement on unresolved matters.

**Part A** of the document provides interpretation of ISO/IEC 17025 for certain aspects of a horseracing laboratory's operation. It does not cover all the requirements of ISO/IEC 17025, with which all laboratories accredited to ISO/IEC 17025, including horseracing laboratories, must comply.

**Part B** contains recommendations for establishing the presence of prohibited substances in the materials tested. Horseracing laboratories should normally comply with these.

**Part C** contains the following recommendations: (i) compliance with an appropriate performance specification as required by the relevant authority; and (ii) adoption of harmonised definitions for terms commonly used by racing chemists.

REFERENCES

*ISO/IEC 17025:2005* General requirement for the competence of testing and calibration laboratories

*ILAC-G18: 04/2010* Guideline for the formulation of scopes of accreditation for laboratories
PART A: INTERPRETATION OF ISO/IEC 17025

The following requirements must be met by all horseracing laboratories accredited to the requirements of ISO/IEC 17025:

1. The laboratory must have measures to ensure that incidences of ‘false-negative’ results are kept to a minimum. These should include:
   - an exchange programme with other similar testing laboratories for cross-checking negative samples, or failing this, blind re-submission of a percentage of negative samples into the analytical system.
   - blind submission of spiked samples or known positive samples into the analytical system.
   [Ref: ISO/IEC 17025:2005, Clause 5.9]

2. Every analytical batch must be accompanied by quality-control measures which will include analysis of appropriate blank(s), calibration of instrument performance parameters using suitably selected chemical standards and, where appropriate, recovery of spiked controls in a representative matrix.
   [Ref: ISO/IEC 17025:2005, Clause 5.9]

3. The storage and handling of controlled drugs must comply with local legislation.
   [Ref: ISO/IEC 17025:2005, Clause 1.5]

4. The laboratory must document the minimum schedule of screening tests to be performed for different types of samples and must also record what tests it has carried out on each sample.
   [Ref: ISO/IEC 17025:2005, Clause 5.4.1]

5. The laboratory must document for each screening test how they decide which samples to investigate further.
   [Ref: ISO/IEC 17025:2005, Clause 5.4.1]

6. Limits of detection for representative analytes must be determined and documented for all screening methods. Compilations must be updated as data accumulates.
   [Ref: ISO/IEC 17025:2005, Clause 5.4.5]

7. All records, including those for negative results, must be checked, preferably by one additional qualified analyst.
   [Ref: ISO/IEC 17025:2005, Clause 5.4.7.1]

These test-method-related requirements are not comprehensive and accreditation bodies may suggest additions to this compilation.
PART B: GUIDE FOR ESTABLISHING THE PRESENCE OF PROHIBITED SUBSTANCES

PREAMBLE

1. This guide was originally adopted by the Association of Official Racing Chemists (AORC) and by laboratory heads connected with the International Federation of Horseracing Authorities and the Association of Racing Commissioners International.

2. The presence of a prohibited substance is established when sufficient valid analytical data supports its presence and no significant data refutes it.

3. The guide provides a set of internationally agreed recommendations for establishing the presence of a prohibited substance, although the concept of rigid standardization is rejected.

4. The guide should not be followed exclusively of other scientific considerations where necessary to establish the presence of a prohibited substance.

5. It is recognized that some laboratories will be able to establish the presence of a wider range of prohibited substances or lower concentrations of prohibited substances than other laboratories. Such individual capabilities must be allowed to develop, as they will lead to improvements generally.

FORENSIC INTEGRITY

6. The sample must have been received, identified, its receipt recorded and then stored under appropriate conditions, all according to the laboratory's documented procedures.

7. Nothing should be introduced into this original sample. If for any reason something (such as a diluent or a washed pipette) must be introduced then a procedure must be followed (such as retaining a portion of that diluent or the pipette washings for future reference) and documented to control for potential contamination.

8. A chain of custody must be maintained and recorded.

8.1 The original sample must be kept securely with only authorized access.

8.2 During tests used as evidence, the partially processed test sample should not be left unattended unless secure with only authorized access.

9. For the analysis of primary or “A” samples, unless the “A” sample is analysed on its own (with controls as detailed in this document), a positive identification or quantification must include analysis of two portions of the original sample. These need not be identical tests but must give consistent findings.

10. All analytical data (including quality control data), data transfers, calculations, chain-of-custody records, and reported conclusions must be verified, preferably by at least one other qualified analyst.
11. The analyst(s) in charge of the work and the analyst(s) verifying the work must be suitably qualified and experienced and able to act as expert witnesses for the purposes of giving evidence.

REGULATORY QUALITATIVE IDENTIFICATION

General Considerations

12. The use of independent, diagnostic data is essential. The detection of prohibited substances should be confirmed by a second technique based on a different analytical principle unless the primary method is accepted as a definitive method. Mass spectrometry or a similarly definitive technique, if applicable to the analyte in question, must be included.

13. A report of a prohibited substance must result from the application of documented test methods to the sample of interest.

13.1 Documented test methods must include procedures for quality control and be validated but they need not be analyte specific.

13.2 Significant deviations from the documented procedure must be recorded.

14. The data record must include evidence of the stability and integrity of the analytical system and the absence of interference between sequentially analysed test samples.

14.1 The concurrent analysis of a system blank (water, buffer, or biological sample free from the analyte in question) is necessary to demonstrate the absence of contamination during analysis. Injection of the system blank should be made immediately before the test sample.

14.2 Elimination of an ‘injector memory’ effect should be demonstrated by injection of a negative control (biological sample or extract negative for the analyte in question) as part of the confirmatory sequence, before the test sample and after any earlier injection which may have contained the analyte in question. Insignificant ‘injector memory’ amounting to less than 2% of the relevant signal from the analyte in the test sample is acceptable.

14.3 Where the analysis of a system blank or negative control is impractical, e.g., for the analysis of Total Carbon Dioxide or other endogenous substances, a control known to contain a lower concentration of the analyte than that present in the test sample may be used instead.

15. Quantification of a sample component is not necessary for a report of a non-threshold substance.

15.1 When quantitative results are a purpose of testing, the additional clauses for regulatory quantification in this document apply.

15.2 A spiked control may be used to establish the required confirmatory detection capability when split-sample verification is part of the jurisdictional process. Appropriate caution must be used and recorded to demonstrate the absence of cross-contamination between the spiked control and the test sample.
15.3 Pharmacologically irrelevant or analytically insignificant levels of certain legitimate therapeutic substances or environmental substances may be present in many test samples. Thus an authority may require the laboratory to control the detection (and reporting) of such non-threshold prohibited substances through the application of internationally-harmonised screening limits (such as http://www.ifhaonline.org/default.asp?section=IABRW&area=1) or internationally-harmonised residue limits (such as www.ifhaonline.org/resources/Feed_Contaminants_Environmental_Substances_Guidelines.pdf).

15.4 Where possible the AORC Guidelines for Controlling the Application of Screening Limits (September 2013 or later version) should be followed, a copy of which can be found on the AORC website under the following link: http://www.aorc-online.org/documents/guidelines-for-controlling-screening-limits/.

16. The identification of a prohibited substance must normally result from direct comparison with a reference material analysed in parallel or series with the test sample.

16.1 The use of library spectra or data other than that generated by a reference material as prescribed would require justification.

16.2 Certified reference materials and reference materials obtained from reference material producers, preferably national metrology institutes or others accredited to ISO Guide 34 by an ILAC Mutual Recognition Arrangement (MRA) signatory or authoritative sources (such as LGC, WHO, The British Pharmacopoeia, The United States Pharmacopeia and other national pharmaceutical authorities), are acceptable after a simple check for its identity or nominal property.

16.3 A reference material is generally accepted for use if it is a chemical with well-established structure, which has been validated in the laboratory by comparison with a certified reference material or by comparison with non-controversial published data or has been structurally characterized.

16.4 An acceptable reference material may also be an isolate from (i) a urine or blood sample after an authenticated administration or (ii) an in-vitro incubation with liver cells, microsomes, plasma or serum, providing the analytical data from it are sufficient to fully justify its identity as a metabolite of the substance administered or incubated.

16.5 An acceptable reference material may also be the product of a chemical transformation of a parent drug or substance by a well-defined and non-controversial chemical method provided that the data from it are sufficient to fully justify its identity.

17. There must be written laboratory criteria for what constitutes a ‘match’ between a reference material and a sample component.

Validation for Qualitative Identification Methods

18. Validation studies are required for the qualitative screening and confirmation methods used and can be conducted by the scientific community (as in the case of standard or published methods) or by the laboratory itself (as in the case of in-house or modified methods).
Screening methods cannot be used alone without conducting a definitive confirmation for any substance to be qualitatively identified and reported.

19. When a method has been validated elsewhere, the laboratory shall ensure that the validation performed was fit for the intended purpose, and shall conduct and document a verification to demonstrate its competency in performing the method.

20. Validation should provide objective evidence that factors that can influence the test results (such as limits of detection and specificity/selectivity for representative analytes) have been assessed and documented, and that the method is reliable and fit for the intended purpose.

21. As the scope of analytes can be very large and reference materials with known quantitative information are not always available, limits of detection do not have to be determined for all analytes in a multi-target screening test. Similarly, the limit of confirmation does not have to be determined for every analyte in a qualitative confirmation test. Measurement uncertainties need not be determined for qualitative screening and confirmation methods.

**Generic Criteria for Common Techniques**

22. Mass spectrometry

22.1 The performance of the mass spectrometer, including accuracy of the mass assignment, ion resolution and (except for tandem mass spectrometry) isotopic abundance, must be determined and recorded within the time frame of the sample analysis using appropriate mass-spectrometric calibration standard(s) or reference material(s).

22.2 The laboratory must document the mass-spectral agreement that the component of interest in the test sample must have in common with the reference material. For full-scan techniques, the base peak and molecular or quasimolecular ion if present should be included.

22.3 Single or averaged spectra or reconstructed ion chromatograms are acceptable for measuring ion-intensity ratios.

22.4 Full-scan data is preferred over selected-ion monitoring (SIM) or selected-reaction monitoring (SRM), since co-eluting interfering substances can be more readily recognized and dealt with.

22.5 SIM or SRM has use where full-scan collection is not applicable or possible due to matrix interference or where quantification is necessary.

22.6 Use of SIM or SRM instead of full scan should be defensible, especially given the widespread use of multiple SRM in qualitative identification of substances in complex matrices. When using SIM or SRM, specific and significant ion(s) or transition(s) must be monitored to ensure proper forensic identification when the data is considered along with data provided by other analytical techniques. The signal-to-noise ratio must be greater than a specified limit.

22.7 Where relevant, the AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry (January 2015 or later version) should be
23. Gas or liquid chromatography

23.1 The retention time (or relative retention time) of the component of interest in the test sample must agree within a specified retention-time window with that of the reference material. The retention-time window should be commensurate with the resolving power of the chromatographic system.

23.2 Where relevant, the AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry (January 2015 or later version) should be followed, a copy of which can be found on the AORC website under the following link: http://www.aorc-online.org/AORC MS Criteria.pdf.

24. Thin-layer chromatography

24.1 The $R_f$ of the component of interest in the test sample must agree within a specified limit with the $R_f$ of the reference material run on the same plate. The reference material should be run either side of the test sample.

24.2 The component of interest in the test sample must respond consistently with the reference material to methods used for locating them.

24.3 This technique alone can be used in screening but not in confirmation (qualitative identification).

25. Immunoassays

25.1 Immunoassay tests must be characterized for detection limits, reproducibility, and specificity.

25.2 A spiked control (or administration control) and a negative control must be included with each set of test samples to ensure proper test performance.

25.3 Instrumental readouts for immunoassay tests are necessary for quantitative or semi-quantitative measurements.

25.4 The documented test methods must define levels that result in acceptably low proportions of unconfirmable hits (these levels must not be construed as official thresholds).

25.5 Immunoassay alone can be used in screening but not in confirmation (qualitative identification).

REGULATORY QUANTIFICATION

26. Equipment

26.1 The equipment must be appropriate for the desired objective and purpose of measurement.
26.2 Apparatus for measuring simple physical parameters such as weight, volume and temperature must be calibrated/checked to a degree commensurate with the required accuracy of the final result.

26.3 Such calibrations/checks must be performed by, or be traceable to reference standards calibrated by, (i) a national metrology institute that is a signatory to the Comité International des Poids et Mesures (CIPM) MRA, or (ii) a calibration laboratory accredited to ISO/IEC 17025 by an ILAC MRA signatory and whose scope of accreditation specifically identifies the appropriate calibration.

26.4 All analytical equipment must have documented calibration and maintenance schedules and no equipment should be used for measurement beyond its calibration time interval.

27. Method

27.1 The method should be robust to variations in the matrix and experimental conditions. Tolerances where critical must be specified.

27.2 The method must be clearly documented. Significant deviations from the documented procedure must be recorded.

27.3 A range of calibration standards prepared in an appropriate matrix should be analysed concurrently with test samples and the data must be recorded.

27.4 The calibration range should be appropriate to the analysis. A zero-level sample must be included as a system blank where practical.

27.5 Measurands (such as Total Carbon Dioxide) with empirical thresholds established by a specific method must be determined by the same method. A second analytical technique may not be necessary to identify its presence in a sample.

28. Internal standards

28.1 Internal-standard techniques are preferable for methods based on extraction then chromatography, although other quantitative techniques are acceptable.

28.2 The internal standard should be added as early in the procedure as possible.

28.3 The internal standard must be of appropriate purity.

28.4 The internal standard should have similar chemical and physical properties to the analyte of interest. Isotopically labelled analytes are the preferred internal standards where quantification is by mass spectrometry.

28.5 The internal standard should be essentially stable to the analytical procedure.
29. Reference materials

29.1 The purity of certified reference materials can be accepted as stated by the reference material producer, if due regard is paid to all handling recommendations.

29.2 The purity of other reference materials must be thoroughly established by:
- comparison with a certified reference material of known purity, or
- checking the supplier's data by analysis, or
- analysis by more than one technique.

29.3 Suppliers' storage and shelf-life information should be paid due regard, and materials checked for stability after prolonged storage.

30. Validation

30.1 The suitability of the method must be demonstrated by acceptable and defensible recorded validation data.

30.2 The laboratory must be able to substantiate that the data is specific to the threshold substance.

30.3 Sample carryover must be demonstrated to be insignificant.

30.4 Validation should characterize trueness and precision.

30.5 The detection limit should be determined as part of the validation if close to or higher than the threshold.

30.6 The laboratory must determine and document its procedure for the estimation of the measurement uncertainty (MU) and the level of confidence associated with the MU.

30.7 The measurement uncertainty (MU) should preferably be determined by recognized methods at or around the threshold or the limit of quantification if this is higher than the threshold. A threshold is then considered exceeded with the stated level of confidence when the determined value in the sample exceeds the threshold plus the MU. Alternatively, MU may be estimated at or around the particular value determined in a sample, and a threshold is considered exceeded with the stated level of confidence when the determined value minus the MU exceeds the set threshold.

31. Quality control

31.1 Samples should be analysed at least in duplicate.

31.2 The stability of stock solutions of reference materials should be known.

31.3 Separately weighed reference material must be used to prepare the stock solutions for the calibration standards and quality controls.

31.4 Quality controls at appropriate concentrations should be analysed concurrently with test samples.

31.5 Criteria for acceptable quality-control results should be determined and documented.
32. Provisional thresholds

32.1 Some thresholds may not be absolute quantities or ratios but a specification agreed with the racing authority, and not all the clauses in this ‘Regulatory Quantification’ section may apply.

REFEREE ANALYSIS

33. The objective of the referee analysis (also known as B-sample analysis or split-sample analysis) is to ensure that the findings of the first analysis are correct by conducting a confirmatory analysis for the presence of the reported substance(s) on the split or remaining portion of the sample, whenever possible by an independent laboratory accredited to the requirements of ISO/IEC 17025.

34. Referee analysis is not intended to be a new analysis requiring screening and confirmatory testing for unnamed substances.

35. Where possible the AORC Guidelines for Referee Analysis (March 2008 or later version) should be followed, a copy of which can be found on the AORC website under the following link: [http://www.aorc-online.org/AORC Referee Guidelines.pdf](http://www.aorc-online.org/AORC Referee Guidelines.pdf).
PART C: ADDITIONAL RECOMMENDATIONS

PERFORMANCE SPECIFICATION

Authorities who are signatories to the relevant articles of the International Agreement on Breeding, Racing and Wagering (IABRW) of the International Federation of Horseracing Authorities (IFHA) should expect their horseracing laboratories to seek accreditation on the basis that they can reliably meet the performance specification adopted by the IFHA. This specification is listed as Performance Specification of the Laboratories for Doping Control Required by the International Federation of Horseracing Authorities, of the IABRW (March 2015 or later version; Clause 18 of Article 6A) and can be found on the IFHA website under the following link: http://www.horseracingintfed.com/default.asp?section=IABRW&area=7.

Other performance specifications that a horseracing laboratory may seek accreditation to include:

(i) the “AORC Proficiency Testing Drug List” of the Proficiency Testing Program Protocol of the Association of Official Racing Chemists (AORC). The current version can be found on the AORC website under the following link: http://www.aorc-online.org/documents/aorc-urine-and-plasma-proficiency-testing-drug-list-2015/; and

(ii) the “Proficiency Testing Parameters” of the Canadian Pari-Mutuel Agency Reference & Research Laboratory, which can be found in: http://palcan.scc.ca/specs/pdf/564_e.pdf.

Where an authority uses a performance specification that differs from any of the above, its horseracing laboratory is required to reliably meet that performance specification.

HARMONISED DEFINITIONS FOR TERMS COMMONLY USED BY RACING CHEMISTS

In order to avoid misunderstanding and confusion, it is recommended that harmonised definitions be adopted for terms commonly used by racing chemists and which are specific to this discipline. The AORC document “A Glossary of Terms Commonly Used in Racing Chemistry” can be found on the AORC website under the following link: http://www.aorc-online.org/AORC Glossary.pdf.