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Accreditation Requirements and Operating Criteria for Horseracing Laboratories

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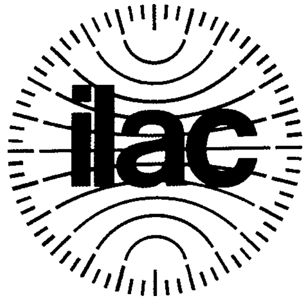

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PREAMBLE

The document *Accreditation requirements and operating criteria for horseracing laboratories* was endorsed for publication by ILAC (now the International Laboratory Accreditation Cooperation) in October 1994 (Resolution 19/94) and has been updated since.

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**: The performance specification for horseracing laboratories that has been adopted by the International Federation of Horseracing Authorities.

RECOMMENDATIONS TO ACCREDITATION BODIES

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

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

Suggestions on Part B would be welcome and should be sent to:

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With reference to the document's Part C, 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:

Field of testing	Chemical testing, *or* Forensic testing
Materials tested	Equine and canine body fluids, *or* Body fluids, tissue, and excreta from animals; materials that an animal may have received or may have been intended to receive
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
Techniques used	Inhouse 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.

AUTHORSHIP

This document was put together by an ILAC working group, convened by Dr D L Crone. **Part A** (accreditation requirements) was compiled by the working group. **Parts B** and **C** (operating criteria) were prepared by the horseracing industry.

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INTRODUCTION

The general requirements for accreditation of laboratories are laid down in ISO/IEC Guide 25, *General requirements for the competence of calibration and testing laboratories*. These requirements apply to all types of calibration and objective testing but need amplification in certain cases.

This document's **Part A** amplifies some of the requirements of ISO/IEC Guide 25 for horseracing laboratories, and **Parts B** and **C** detail operating criteria that should normally be adopted.

Where there are differences of interpretation, ISO/IEC Guide 25 is the authoritative document, and individual accreditation bodies will adjudicate on unresolved matters.

Part A of the document deals with the amplification of ISO/IEC Guide 25 for certain aspects of a horseracing laboratory's operation. It does not cover all the requirements of ISO/IEC Guide 25, with which all laboratories including horseracing laboratories must comply.

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

Part C is the performance specification adopted by the International Federation of Horseracing Authorities. It is expected that most horseracing laboratories under that umbrella will seek accreditation on the basis that they can demonstrate that they meet this specification reliably. Some, however, may seek accreditation to a different performance level, particularly one specified by a client.

PART A: INTERPRETATION OF ISO/IEC GUIDE 25

The following requirements must be met by all horseracing laboratories operating to ISO/IEC Guide 25:

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 negative samples into the analytical system
   - blind submission of spiked samples or known positive samples into the analytical system.
     [Ref: ISO/IEC Guide 25:1990, Clause 5.6]

2. Every analytical batch must be accompanied by quality-control measures which will include analysis of a system blank, calibration of instrument performance parameters by suitably selected chemical standards, and where appropriate, recovery of spiked control samples with a representative matrix.
   [Ref: ISO/IEC Guide 25:1990, Clause 5.6]

3. The storage and handling of controlled drugs must comply with local legislation.
   [Ref: ISO/IEC Guide 25:1990, note to Clause 7]

4. The initial screening procedure for prohibited substances must specify the minimum schedule of tests for samples to be reported as negative.
   [Ref: ISO/IEC Guide 25:1990, Clause 10.1]

5. The laboratory must document for each screening test how they decide which samples to investigate further.
   [Ref: ISO/IEC Guide 25:1990, Clause 10.1]

6. Limits of detection for representative analytes must be determined and documented for all methods. Compilations must be updated as data accumulates.
   [Ref: ISO/IEC Guide 25:1990, Clause 10.4]

7. All records, including those for negative results, must be checked.
   [Ref: ISO/IEC Guide 25:1990, Clause 10.6]

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

(Part B has also been issued separately as Guide for establishing the presence of prohibited substances*, Issue 2, November 1996.)*

PREAMBLE

1. This guide has been adopted by the Association of Official Racing Chemists 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, and the receipt recorded, then the sample stored under appropriate conditions, all according to the laboratory's documented procedures.

7. Nothing must be introduced into this original sample without stringent documented controls.

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

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

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

9. Unless the 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), manual data transfers, calculations, chain-of-custody records, and reported conclusions must be verified.

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 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. 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 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 samples.

    14.1 The concomitant 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 should be immediately before the test sample.

    14.2 Elimination of an 'injector memory' effect should be demonstrated by injection of a negative control sample (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.

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 sample 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.

16. The identification of a prohibited substance must 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 are obtainable from national and international institutes and certifying bodies of an equivalent status (such as USP, BP, WHO, and other pharmaceutical authorities) and are acceptable after a simple check for identity.

    16.3 A reference material is generally accepted as 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 A reference material may also be an isolate from a urine or blood sample after an authenticated administration, providing the analytical data from it is sufficient to fully justify its identity as a metabolite of the substance administered.

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

Generic Criteria for Common Techniques

18. Mass spectrometry

18.1 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).

18.2 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 pseudomolecular ion if present should be included.

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

18.4 Full-scan data is preferred over selected-ion monitoring, since co-eluting interfering substances can be more readily recognized and dealt with.

18.5 Selected-ion monitoring has use where full-scan collection is not applicable or where quantification is necessary.

18.6 Use of selected-ion monitoring instead of full scan should be defensible. When using selected-ion monitoring, specific and significant ion(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.

19. Gas or liquid chromatography

19.1 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.

20. Thin-layer chromatography

20.1 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.

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

21. Immunoassays

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

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

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

21.4 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.)

22. Ultraviolet or fluorescence spectroscopy

22.1 The spectrum of the component of interest in the test sample must agree within specified limits with that of the reference material. The wavelength maxima should agree within a margin commensurate with the resolution of the instrument.

REGULATORY QUANTIFICATION

23. Equipment

23.1 The equipment must be appropriate for the desired objective and purpose of measurement.

23.2 Apparatus for measuring simple physical parameters, such as weight, volume, temperature, must be calibrated to a degree commensurate with the required accuracy of the final result.

23.3 Such calibrations must be traceable to national standards of measurement.

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

24. Method

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

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

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

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

25. Internal standards

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

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

25.3 The internal standard must be of appropriate purity.

25.4 The internal standard should preferably be isotopically labelled analyte where quantification is by mass spectrometry.

25.5 The internal standard should be essentially stable to the analytical procedure.

26. Reference materials

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

26.2 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.

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

27. Validation

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

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

27.3 Sample carryover must be demonstrated to be insignificant.

27.4 Validation should characterize accuracy.

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

27.6 The laboratory must determine and document its policy on uncertainty of measurement.

27.7 The uncertainty of measurement should be determined by recognized statistical methods using controls at or around the threshold or the limit of quantification if this is higher than the threshold.

28. **Quality control**

28.1 Samples should be analysed at least in duplicate.

28.2 The stability of stock solutions should be known.

28.3 Separately weighed reference material must be used to prepare the stock solutions for the calibration standards and quality-control samples.

28.4 Quality-control samples at appropriate concentrations should be analysed concurrently with test samples.

28.5 Criteria for acceptable quality-control results should be determined and documented.

29. **Provisional thresholds**

29.1 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.

PART C: PERFORMANCE SPECIFICATION OF THE INTERNATIONAL FEDERATION OF HORSERACING AUTHORITIES

(Part C has also been issued separately as Performance specification for racing laboratories*, Issue 1, October 1993.)*

This specification describes the minimum routine analytical capability required of racing laboratories. It must not be construed as restricting in any way the chemical groups or concentrations that may constitute positive findings. Many laboratories will be able to surpass this specification, and the interpretation of whether a finding constitutes a positive finding is governed by the Rules of Racing.

Procedures should ensure that the laboratory can find prohibited substances in equine body fluids and prove their presence. Test methods must cover the chemical groups typified in the representative list. Each substance in the representative list must be detectable and identifiable at the concentration shown, if necessary after hydrolysis of metabolic conjugates.

Representative list	**ng per mL**
Acepromazine	100
Amphetamine	500
Benzocaine	1000
Benzoylecgonine	200
Boldenone	100
Butorphanol	200
Caffeine	100
Dexamethasone	50
Flufenamic acid	500
Flunixin	2000
Frusemide	2000
Hydrochlorothiazide	2000
Imipramine	100
Lignocaine	100
Methylphenidate	200
Nordazepam	1000
Oxprenolol	400
Pentobarbitone	2000
Phenylbutazone	2000
Theophylline	1000

Test methods must also reliably quantify threshold substances at values that would be declared positive.