ANVISA's regulatory perspective on progress in alternative method acceptance

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Conference on animal testing replacement for vaccines
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RESOLUTION RDC Nº 55, OF DECEMBER 16, 2010

When submitting an application for registration of a biological product, complete data proving the quality, efficacy and safety of the product must be presented.

Quality control

- description of quality control tests;
- validation of analytical methodologies;
- reference standards;
- justification for specifications.

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RESOLUTION Nº 948, OF DECEMBER 12, 2024

Provides for the health requirements for the regularization of medicines for human use.

Quality control tests for active pharmaceutical product and finished products may use official pharmacopoeial methods or validated in-house development methods, provided they are duly validated under the terms of the Resolution RDC No. 166, of July 24, 2017, and its updates.

The pharmacopoeial methods contained in the following are considered official:
I - Brazilian Pharmacopoeia (FB), as per Resolution RDC No. 940, of November 14, 2024, and its updates; and
II - recognized Pharmacopoeias, as per Resolution RDC No. 511, of May 27, 2021, and its updates.

In-house development methods must be equivalent to or superior to those of the FB, if any.

In-house development methods may be accessed, evaluated, and incorporated by the FB, unless expressly refused by the company holding the regularization.

The finished product must meet the tests and acceptance criteria of the specific monographs and applicable general methods of the current FB, even if it adopts a different quality control analysis method. It will be tested following the official monograph by official national laboratories.

When FB's tests and acceptance criteria are not suitable for the product, the company must provide a justification based on technical and scientific criteria.

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Pharmacopoeial methods ⇆ *In-house* methods

In-house methods: complete validation and equivalence or superiority to the pharmacopoeial method.

Some important points:

- Comparable method sensitivity;
- Scientific evidence;
- Comparison of historical results with in-house method results;
- Justification for the lack of a correlation between *in vitro* tests and *in vivo* responses, when applicable;
- Batches that fail the approved test must also be rejected by the in-house test;
- *In vitro* tests are recommended to replace *in vivo* tests;
- *In vitro* tests should reflect human biology.

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RESOLUTION-RDC Nº 741, OF AUGUST 10, 2022

Admissibility of analysis carried out by an Equivalent Foreign Regulatory Authority (AREE).

- AREE analysis admitted for the adoption of an optimized analysis procedure facilitated by regulatory trust practices;
- Recognition: a practice of regulatory trust, in which the decision of another regulatory authority or international entity may be adopted by Anvisa;
- Collaborative work: a practice of regulatory trust, in which two or more regulatory authorities share activities to carry out a specific regulatory task;
- Regulatory reliance enables accelerated implementation of globally validated and scientific based non-animal testing approaches.

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NORMATIVE INSTRUCTION - IN Nº 289, OF MARCH 20, 2024

Sets the criteria applied to the optimized analysis procedure that uses the assessments conducted by AREE to analyze petitions.

- IN nº 289 complements the Resolution 741/2022;
- Verified analysis: verification of the applicability of the results of the assessment of an equivalent foreign regulatory authority (AREE), for regulatory decision-making in the national context;
- Supporting documentation issued by an AREE to which the regularization request was submitted and approved must be filed;
- The technical and legal documentation must meet all the requirements, criteria and specifications established by Anvisa for registration or post-registration (RDC nº 55/2010 or RDC nº 913/2024);
- When a technical parameter is approved in an AREE, it is usually considered as a point already harmonized in the technical analysis;
- Typically, the rationale accepted by AREE for substitution of *in vivo* testing is accepted in optimized analysis procedures;
- Regulatory reliance is one way to accelerate the acceptance of alternative methods.

Brazilian's commitment to the 3 R's principle

Constitution of the Federative Republic of Brazil of 1988

Art. 225. Everyone has the right to an ecologically balanced environment, a common good for the people and essential to a healthy quality of life. Government and the community have the duty to defend and preserve it for present and future generations.

§ 1 To ensure the effectiveness of this right, it is the responsibility of the Government to:

(...)

VII - protect fauna and flora, prohibiting, as provided by law, practices that endanger their ecological function, cause the extinction of species, or subject animals to cruelty.

(...)

Law nº 11794/2008

Art. 1. The breeding and use of animals in teaching and scientific research activities, throughout the national territory, must comply with the criteria established in this Law.

(...)

§ 2. All activities related to basic science, applied science, technological development, production and quality control of drugs, medicines, food, immunobiologicals, instruments, or any other activities tested on animals, as defined in specific regulations, are considered scientific research activities.

(...)

Art. 4. The National Council for the Control of Animal Experimentation – CONCEA is hereby created.

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National Council for Animal Control and Experimentation (CONCEA)

Anvisa Resolution RDC nº 35/2015
- Acceptance of alternative methods to the use of animals in petitions submitted for analysis through CONCEA;
- Exceptions are made in specific cases where Anvisa, through technical justification, demonstrates the inadequacy and inapplicability of methods recognized by CONCEA.

CONCEA Normative Resolution nº 45/2019
- Monocyte Activation Test (MAT) recognition for assessing pyrogenic contamination in injectable products;
- Alternative method validated by international validation centers and with international regulatory acceptance;
- A period of up to 5 (five) years for mandatory replacement of the original method with the alternative method.

CONCEA Public Consultation Notice nº 60/2025
- Recognition of the Recombinant Factor C (rFC) method for detection of bacterial endotoxins;
- Formally validated by international studies and has international regulatory acceptance;
- Term of up to 05 (five) years as the limit for the mandatory replacement of the endotoxin method by Limulus Amebocyte Lysate (LAL)

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Brazilian Pharmacopoeia 7th Edition - 2024

- Inclusion of the Monocyte Activation Test (MAT): an important step towards the adoption of alternative methods to the use of animals;
  - (5.5.2.7) PYROGEN EVALUATION TEST
  - Inclusion of an alternative pyrogen determination method to the rabbit test;
  - MAT is the test of choice for evaluating total pyrogens, aiming to reduce the use of animals.
- Exclusion of an *in vivo* toxicity test from the General Methods;
- Commitment of the Brazilian Pharmacopoeia to more ethical and sustainable practices.

Monocyte Activation Test (MAT)

- **Brazilian Pharmacopoeia 7th Edition**

(5.5.2.7) PYROGEN EVALUATION TEST

(5.5.2.7.1) MONOCYTE ACTIVATION TEST
- Test of choice for detecting total pyrogens;
- Product-specific verification.

(5.5.2.7.2) RABBIT PYROGEN TEST
Use of rabbit pyrogen test only when:
- Proven mitigation of the risk of pyrogen contamination;
- Sample interference in the MAT makes its application unfeasible.

(5.5.2.7.3) BACTERIAL ENDOTOXIN TEST
Can replace total pyrogen testing:
- Product specific risk analysis to highlight the low risk of non-endotoxin pyrogen (NEP) in the sample;
- Correlation of results obtained using the bacterial endotoxin method and a total pyrogen methods.

- Other pyrogen detection methods approved in official compendia recognized by current Anvisa legislation.

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Thematic Technical Committee for Biological Products of the Brazilian Pharmacopoeia

- Public Consultation nº 1.342, August 11, 2025
  - Revised monographs of vaccines for human use, yellow fever vaccine (attenuated) and rabies vaccine (inactivated);
  - Pharmacopoeial texts harmonizad with the guides of the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), World Health Organization (WHO) and recognized international Pharmacopoeias;
  - Wherever possible and supported by scientific evidence, advanced and robust alternative methods were included.
- Expected Public Consultation
  - Revised measles and varicella monograph.
- Development of Recombinant Factor C (rFC) method

Vaccines for human use

- **Main proposed changes**
  - **Control of adventitious agents**
    - Control of adventitious agents in viral vaccines should be based on a risk analysis;
    - *In vitro* tests and molecular biology tests with high sensitivity and broad detection capacity are recommended;
    - *In vivo* tests should only be used when their use is proven to mitigate the risk of contamination by potential adventitious viral agents.
  - **Quality control**
    - Recommendation for the use of alternative tests to *in vivo* tests;
    - Alternative methods validated against *in vivo* methods;
    - If data from two analytical procedures cannot be statistically compared, the conclusion on comparability will be based on evidence that the results of the alternative and the *in vivo* method pass/fail for the same samples;
    - For potency methods, if the impossibility of performing comparative validation between the alternative and the *in vivo* method cannot be justified, the alternative method must reflect both the content and functionality of the epitope (native conformation) relevant to the protection offered by the vaccine;
    - For potency methods, the alternative method must demonstrate its ability to differentiate samples of different concentrations and to discriminate between potent and subpotent batches;
    - The alternative test must demonstrate concordance with the immune response in humans.

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Yellow fever vaccine (attenuated)

- **Main proposed changes**

  - **Master and working seed lots:**
    - adventitious agents tests according to the *Vaccines for Human Use* monograph;
    - *in vitro* tests and molecular biology tests with high sensitivity and broad detection capacity are recommended.

Rabies vaccine (inactivated)

- **Main proposed changes**

  - **Residual infectious virus test:**
    - *In vivo* tests excluded;
    - *In vitro* test with detection by immunofluorescence or other method of equivalent sensitivity included.

  - **Immunogenic activity determination assay:**
    - Option to reduce the control group's mice population from 30 to 15-30;

    - Application of alternative parameters:
      - Anesthesia prior to challenge;
      - Humane outcomes in animal observation rather than fatal outcomes to reduce animal suffering;

    - The use of validated alternative methods, replacing the *in vivo* method, is recommended, according to the Vaccines for Human Use monograph;

    - Immunoenzymatic methods must employ extensively characterized rabies virus-neutralizing monoclonal antibodies directed against the rabies virus G glycoprotein in its native (trimeric) conformation.

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Thank you!

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