Welcome

Empowering a healthy tomorrow

Alternate Pyrogen Tests

Radhakrishna S.Tirumalai, Ph.D.
Principal Scientific Liaison-General Chapters
US Pharmacopeial Convention
Rockville, MD. U.S.A.

Pyrogens

- Substances inducing fever
- Biological events
  - Levels of circulating inflammatory cytokines (IL-1, IL-6, TNF-α)
- Clinical events
  - Fever
  - Hypotension
  - Lymphopenia, Neutrophilia
- High Doses – Septic Shock

Pyrogens

CLASSIFICATIONS:

- Exogenous – Substances that originate in the environment
- Endogenous – Substance(s) produced internally by the host in direct response to stimulus from exogenous pyrogens

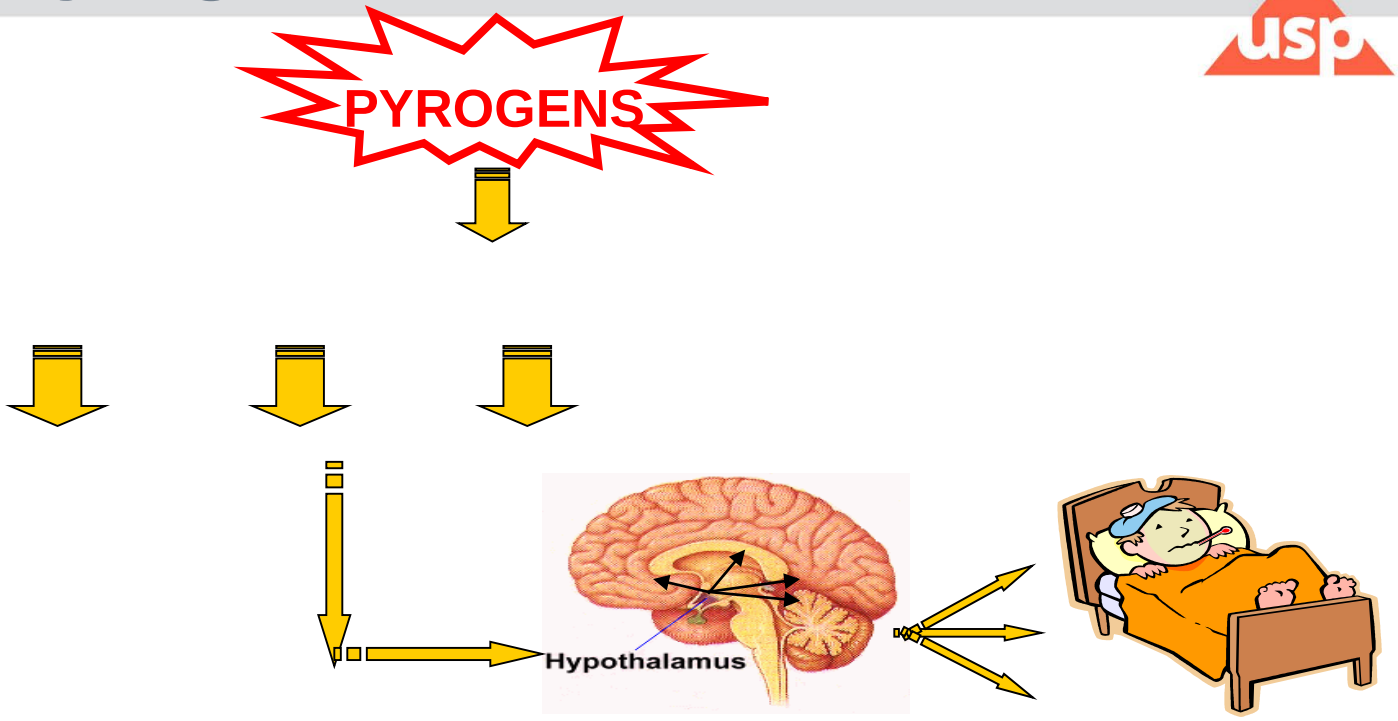
Exogenous Pyrogens

- Endotoxins (Gram-negative bacteria)
- Viruses
- Fungi (mannans, glucans)
- Cell wall components
  - Lipoteichoic acids
  - Peptidoglycans
  - Muramylpeptides
  - Porins
- Exotoxin A
- Polynucleotides (poly I : poly C)

Endogenous Pyrogens

- Proinflammatory Cytokines
  - IL-1
  - IL-6
  - TNF-α
- Prostaglandins

In Vivo Pyrogenic Reactions



Pyrogen

A pyrogen is:

any chemical substance that will cause a fever if present at high enough levels within the body.

Pyrogen Testing <151>

Pyrogens

- A pyrogen is a substance that induces fever when injected into human subjects.
- The pyrogen test depends on the similar pyrogenic thresholds in humans and rabbits.

History of the Pyrogen Test

- In investigations of “injection fever”, Hort and Penfold (publishing in 1912) developed a rabbit pyrogen test.
- Demonstrated that Gram negative bacteria were pyrogenic whereas Gram-positive organisms were not.
- Seibert demonstrated that injection fevers were caused by filterable, heat stable pyrogens preparations from gram negative bacteria.
- Seibert’s work resulted in (unofficial) rabbit pyrogen tests being used in quality control of large volume parenteral solutions.

Advent of the USP Pyrogen Test

- World War II lead to a heavy demand for IV therapy.
- The need for a standardized, official (USP) test became apparent.
- Co Tui (1942) concluded that rabbits were the animal of choice for the demonstration of the absence of pyrogens (despite some false positive results).
- A collaborative study led by Welch in 1941 (published in 1943), which involved 14 pharmaceutical manufacturers and 3,300 pyrogen tests.
- Resulted in the first pyrogen test procedure in USP XII in 1942.

Pyrogen Testing

The USP Pyrogen Test <151> is an in vivo test for the presence of pyrogens

- It is designed to limit to an acceptable level the amount of pyrogens within an injectable pharmaceutical
- The USP Pyrogen Test is a limit test!

Pyrogen Test <151>

- It is designed to test pharmaceutical injectables

- It can be adapted to test medical devices that are meant to come in contact with body surfaces or implanted. (See General Chapter <161>)
  - Washes/rinses from devices are injected into the rabbits

<161> Medical Devices-Bacterial Endotoxins and Pyrogen Tests

- The methods and requirements in this chapter apply to assemblies or devices labeled sterile and nonpyrogenic that are in contact directly or indirectly with the cardiovascular system, lymphatic system, or cerebrospinal fluid. This includes, but may not be limited to, the following:
- Fluid pathways of catheters and administration sets such as solution administration sets, extension sets, transfer sets, blood administration sets, intravenous catheters, implants, extracorporeal oxygenator tubing, dialysis tubing, intramuscular drug delivery catheters, and transfusion and infusion assemblies
- Liquid medical devices such as dialysate
- Implantable medical devices such as heart valves and vascular grafts, and other medical devices with a nonpyrogenic claim that may come into contact with blood or cerebrospinal fluid
- Gels with a nonpyrogenic claim including demineralized bone matrices and drug delivery systems

PYROGENS

- For samples that cannot be tested by BET because of non-removable inhibition or enhancement of the test, <151> is applied.

General Notes (not from the USP)

- There is no pyrogen limit (amount of pyrogen per unit of product) analogous to an endotoxin limit.
- For a product to meet the requirement of the test, a negative test is required.

  i.e. no pyrogenic response at the specified dose.

Pyrogen Test

Disadvantages:

- Animal based test
- Expensive
- Non-quantitative
- Very sensitive to animal strain
- Very sensitive to physiological state of test animal
- Very sensitive to stress level of test animal

What is an alternative to Pyrogen test <151>?

Pyrogen Test <151>: Alternatives

Bacterial Endotoxins Test <85>

Why?

Most pyrogens of pharmaceutical concerns are bacterial endotoxins

USP's General View on the Use of Alternative Methods

USP General Notices 6.30:

"An alternative method or procedure is defined as any method or procedure other than the compendial method or procedure for the article in question. The alternative method or procedure must be fully validated (see Validation of Compendial Procedures ⟨1225⟩) and must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis. Alternative methods or procedures can be developed for any one of a number of reasons not limited to simplification of sample preparation, enhanced precision and accuracy, improved (shortened) run time, or being better suited to automation than the compendial method or procedure.USP41 Only those results obtained by the methods and procedures given in the compendia are conclusive

Methods in USP

- **Qualitative** - Is something there?
  - <71> *Sterility Tests, <151> Pyrogen Tests, <85> BET-Gel Clot Limit Test*
- **Quantitative** - How many are there?
  - <61> *Enumeration Tests, <85> BET-Gel Clot Assay and Photometric Tests*
- **ID** - What is there?
  - <62> *Tests for Specified Microorganisms*

Parameters to Address in Quantitative Methods

- Accuracy
- Precision
- Specificity
- Limit of quantification
- Linearity
- Limit of Detection
- Range
- Ruggedness
- Robustness

Performing a sound validation
Is the reference method a gold standard?

Option	Demonstration	Comparison to Offficial Compendial Method	Based on Numerical Results or Conclusion	Number of Characteristics
Acceptable procedures	Acceptable	No	Results	Multiple
Performance equivalent	Equivalent	Yes	Results	Multiple
Results equivalent	Equivalent	Yes	Results	Single
Decision equivalent	Equivalent	Yes	Conclusions	Single

The evolution of new referee methods

- The USP is open to the inclusion of new referee test methods.
- As technology advances evaluation of candidate analytical methods that might supplant existing referee methods is planned.
- Any new referee method must be broad in application, i.e. suitable for use with the vast majority of monographed products.
- Any new candidate referee method must not be single source, patented technology.
- Any new candidate referee method must be open source and able to be applied in any laboratory,

Current Landscape – U.S. documents, CFR

- TITLE 21--FOOD AND DRUGS
- CHAPTER I--FOOD AND DRUG ADMINISTRATION
  DEPARTMENT OF HEALTH AND HUMAN SERVICES
- SUBCHAPTER F--BIOLOGICS
- PART 610 -- GENERAL BIOLOGICAL PRODUCTS STANDARDS
- Subpart B--General Provisions
- Sec. 610.13 Purity

- (b) *Test for pyrogenic substances.* Each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in paragraphs (b) (1) and (2) of this section: *Provided,* That notwithstanding any other provision of Subchapter F of this chapter, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Cryoprecipitate; Plasma; Source Plasma; Normal Horse Serum; bacterial, viral, and rickettsial vaccines and antigens; toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

- (2) *Test procedure, results, and interpretation; standards to be met.* The test for pyrogenic substances shall be performed according to the requirements specified in United States Pharmacopeia XX.

Current Landscape – U.S. documents, FDA

- Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers
  - June 2012
  - Compliance

9. *When is the USP Chapter <151> Pyrogenicity Test (the rabbit pyrogen test) appropriate?*

For certain biological products, 21 CFR 610.13(b) requires a rabbit pyrogen test. The requirement in 21 CFR 610.13(b) may be waived if a method equivalent to the rabbit pyrogen test is demonstrated in accordance with 21 CFR 610.9.

For human and animal drugs, some USP monographs still require a rabbit pyrogen test. Even with such monographs, a firm may substitute an endotoxins test or alternative cell-based test if the firm can demonstrate equivalent pyrogen detection. The appropriate FDA review division will consider alternative methods, such as monocyte activation, on a case-by-case basis.

For devices and drug materials, firms should assess the risk of the presence of non-endotoxin pyrogens. If the risk assessment indicates that non-endotoxin pyrogens may be present, it may be more appropriate to use the rabbit pyrogen test.

Bacterial endotoxins assays are subject to a variety of interferences related to the physical and chemical properties of the test article. Where such interferences cannot be mitigated through sample dilution (up to the MVD) or other validated means of sample preparation, firms should use the rabbit pyrogen test.

Current Landscape – U.S. documents, USP

- ⟨151⟩ PYROGEN TEST
  - INTRODUCTION

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, of the product concerned. The test involves measuring the rise in temperature of rabbits following the intravenous injection of a test solution and is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 mL/kg injected intravenously within a period of not more than 10 min. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph or, in the case of antibiotics or biologics, the additional directions given in the federal regulations.

Influencial input - ICCVAM

- ICCVAM : Interagency Coordinating Committee on the Validation of Alternative Methods
- U.S. Agencies involved:
  - National Institute of Environmental Health Sciences
    - National Institutes of Health
    - U.S. Public Health Service
  - Department of Health and Human Services

- ICCVAM Test Method Evaluation Report, 2008
- “Validation Status of Five In Vitro Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products”

- Used data from European validation studies (European Centre for Validation of Alternative Methods)

Influencial input - ICCVAM

- Based on this evaluation, ICCVAM recommends that, although none of these test methods can be considered a complete replacement for the RPT for all testing situations for the detection of Gram-negative endotoxin, they can be considered for use to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to validation for each specific product to demonstrate equivalence to the RPT, in accordance with applicable U.S. Federal regulations (e.g., U.S. Food and Drug Administration

Awareness of other global thinking

- EP 2.6.8 Pyrogens
- EP 5.1.10 Guidelines For Using The Test For Bacterial Endotoxins
- (refers to 2.6.30 for non-endotoxin pyrogens)
- EP 2.6.30 Monocyte-Activation Test

In Europe MAT can be used after a product specific validation (no equivalence with Rabbit test needed).

Current Landscape – U.S. documents, USP rev.

- ⟨151⟩ PYROGEN TEST
  - INTRODUCTION
    - The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, of the product concerned. The test involves measuring the rise in temperature of rabbits following the intravenous injection of a test solution and is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 mL/kg injected intravenously within a period of not more than 10 min. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph or, in the case of antibiotics or biologics, the additional directions given in the federal regulations (see *Biologics* ⟨1041⟩). ▲A validated, equivalent in vitro pyrogen or bacterial endotoxin test may be used in place of the in vivo rabbit pyrogen test,[1] where appropriate.

Regional thinking, U.S.

- Is Time a factor in choosing what test to perform?
- The LAL test and rFC test are performed in about 2 hours
- A Rabbit pyrogen test is performed in 3 hours (after pre-test preparation)
- The Monocyte Activation Test is performed in 24 hours

Regional thinking, U.S.

- Is Validation a factor in choosing what test to perform?
- Bacterial endotoxin test - product-specific, regulatory approval
- rFactor C test - product-specific, regulatory approval
- Rabbit pyrogen test – nominal
- Monocyte Activation Test - product-specific, regulatory approval

Are there other issues?

- Concerns about what is the standard reference material
  - BET – reference std. endotoxin is used
  - MAT – reference std. or control std. endotoxin can be used for curve
- Standardized blood cells?
  - variability due to human variation
  - supply sustainability
  - determination of quality of supply
  - safety of supply (blood requires unique handling)
- Off the shelf kits
  - Only 1 vendor currently?

Influencing change

- European legal mandate can lead to increased MAT use
- U.S. - Food and cosmetic safety testing use of animals has decreased due to required labeling changes and also consumer reaction to products that utilize this testing in their development
- Pharmaceutical industry in general has not interpreted the change as significant, because LAL is still considered an in vitro test, and extraneous non-endotoxin pyrogens are not commonly found at this point in time across many injectable products
- MDDT
- Education

Future Workshops

- Future of Endotoxins and Pyrogen Testing: Standards and Procedures. June 10-11, 2019, USP-Rockville, Rockville, MD

Questions

Empowering a healthy tomorrow

Thank You

Empowering a healthy tomorrow

Stay Connected

301-816-8339 | rst@usp.org

Empowering a healthy tomorrow