Joint EDQM-EPAA Event

The future of pyrogenicity testing: phasing out the rabbit pyrogen test

14-15 February 2023

Joint EDQM-EPAA Event

The future of pyrogenicity testing: phasing out the rabbit pyrogen test

Regulatory Session

Federal Institute
for Drugs
and Medical Devices

Phasing out the rabbit pyrogen test – the view from the perspective of antibiotics

Dr. Uwe Lipke

Preliminary Remark

This presentation shows my personal view and should not be interpreted as the opinion of the BfArM or any other European Competent Authority.

In particular the new general chapter 5.1.13 “Pyrogenicity” and the changes to the monographs “Parenteral Preparations” and “Substances for Pharmaceutical Use” may lead to differentiated regulatory decisions.

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5. Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?
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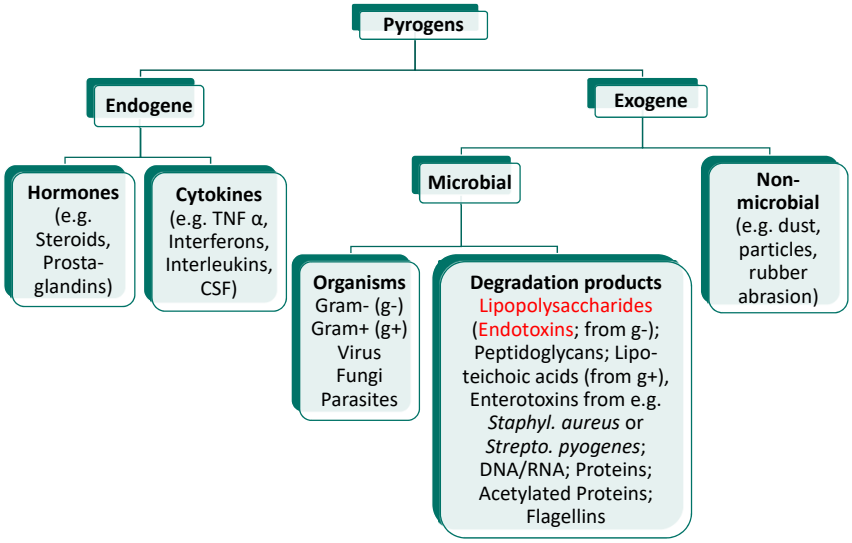
A short overview about pyrogens



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A short overview about pyrogens

How can pyrogens be detected?

- **Rabbit Pyrogene Test (RPT)**
- **Bacterial Endotoxin Test (BET) / Limulus Amebocyte Lysate (LAL) Test**
- **Monocyte Activation Test (MAT)**

Rabbit test (to be phased out in near future)

- Sample is injected and change in body temperature is measured
- Sensitivity depends on experimental conditions, may be lower than in humans
- Detect endotoxin and non-endotoxin pyrogens (NEP)

A short overview about pyrogens

Bacterial Endotoxin Test (BET) / Limulus Amebocyte Lysate (LAL) Test

- Includes Gel Clot, Kinetic or Turbidimetric, Endpoint or Kinetic Chromogenic
- Very sensitive (about 100 times more than RPT for most substances)
- Specific for lipopolysaccharides – NEPs are not detected; however, cross reaction possible with ß-glucans
- Influenced by e.g. complexing agents (EDTA), proteases, and amphiphilic molecules (e.g. Span®; Tween®) in the formulation
- Test for interferences (positive product control) is necessary for each sample
- Newest development: recombinant factor C; no need for Limulus blood, no cross reaction with ß-glucans; however, it detects bacterial endotoxins only
- Nevertheless, animals (the blood from horseshoe crabs) are still needed (except for recombinant factor C, 2.6.32)

A short overview about pyrogens

Monocyte Activation Test (MAT)

- Pyrogens induce production of cytokines (interleukins) in monocytes (corresponding to the fever reaction in humans), measured usually by ELISA
- Sensitivity for lipopolysaccharides lower than if tested with BET
- Detect all pyrogens responsible for fever reaction in humans
- Commercial kits available; however, a product specific validation is necessary

Control of pyrogens in antibiotics – a brief historical summary

- Common test on pyrogenicity in antibiotics was the Rabbit Pyrogen Test
- About 25 years ago, Ph. Eur. third edition (1997) contained several monographs for antibiotics where the RPT was still indicated as test on pyrogens
- Removal of this animal test has long been pursued (since 1986: European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes – “3Rs” – replacement; reduction; refinement)
- With the years, the RPT was replaced by the BET in 49 monographs for antibiotics (according to EDQM review of animal welfare progress 2007)

Control of pyrogens in antibiotics – a brief historical summary

Fermentation products	Semi-synthetic products derived from a fermentation product	
Amphotericin B	Amoxicillin Na	Netilmicin sulphate
Bacitracin	Ampicillin Na	Oxacillin Na H_2O
Benzylpenicillin Na + K	Benzylpenicillin benzathine + procaine	Piperacillin Na
Bleomycin sulphate	Cefalotin Na	K clavulanate
Chlortetracycline HCl	Cefamandole nafate	Sulbactam Na
Cyclosporine	Cefapirin Na	Tiamulin (vet)
Daunorubicin HCl	Cefazolin Na	Ticarcillin Na
Doxorubicin HCl	Cefoperazone Na	
(Fosfomycin Na) – nowadays mostly synthetic	Cefotaxime Na	
Framycetin sulphate	Ceftazidime $5H_2O$	
Gentamicin sulphate	Ceftriaxone Na	
Mitomycin	Cefuroxime Na	
Oxytetracycline HCl	Clindamycin PO_4	
Rifamycin Na	Cloxacillin Na	
Spectinomycin 2HCl $5H_20$ + sulphate $4H_2O$	Dihydrostreptomycin sulphate (vet)	
Streptomycin sulphate	Doxycycline hyclate	
Tetracycline HCl	Epirubicin HCl	
Tobramycin	Imipenem	
Vancomycin HCl	Minocycline HCl $2H_2O$	

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Source: EDQM; Animal Welfare Progress; PA/PH/SG (07) 8

Control of pyrogens in antibiotics – a brief historical summary

- These 49 monographs included fermentation products like benzylpenicillin, chemically synthesised products like fosfomycin, and semi-synthetic products derived from fermentation products like ceftazidime pentahydrate – thus: with different risks for potential presence of NEPs
- I did not find further documents about the validations performed for replacement of RPT by BET for these monographs

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Control of pyrogens in antibiotics – a brief historical summary

Nonetheless, eight monographs for antibiotics still contain the RPT

- Amikacin (semi-synthetic product; 4 chemical steps)
- Chloramphenicol sodium succinate (most likely chemically synthesised nowadays but could be produced semi-synthetically)
- Colistimethate sodium (semi-synthetic product; 2 chemical steps)
- Dicloxacillin sodium (semi-synthetic product; 3 chemical steps)
- Flucloxacillin sodium (semi-synthetic product; 3 chemical steps)
- Kanamycin acid sulfate (fermentation product)
- Kanamycin monosulfate (fermentation product)
- Polymyxin B sulfate (fermentation product)

Removal of RPT from monographs – not that easy

- Group 7 tried to replace the RPT by the BET in two monographs without success
- Flucloxacillin sodium 2012: the provided validation data were considered as not sufficient by the BET WP to justify the replacement of the RPT
- Colistimethate sodium 2016: results of 226 batches produced between 2009 and 2016 (RPT was always negative while 5 batches were positive for BET) were no sufficient evidence for removal of RPT from the monograph as these data were from one manufacturer only – to be of note: that was the sole manufacturer of this substance in Europe at that time
- Results from further manufacturers are not always available for the group of experts – sufficient validation data are difficult to obtain
- On the other hand: BET is now the common test on pyrogens for antibiotics

Removal of RPT from monographs – not that easy

- In order to replace the RPT by the BET in the remaining monographs, evidence is needed that NEPs do not play a role – of course, on substance level and independent of the concrete manufacturing process
- Risk assessment as indicated by current Ph. Eur. 5.1.10 on presence of NEPs is possible for a concrete process only – not independent of it
- Two options seemed to remain for removal of RPT:
  - An adequate number of manufacturers show that BET is sufficient and NEPs can be considered uncommon for this substance; BET to be included
  - Introduction of MAT for the remaining 8 monographs
- If MAT were developed for these 8 monographs, what about the other 49 monographs for antibiotics where RPT has been replaced by BET?

MAT or BET for control of pyrogens in antibiotics?

- Following the failure with flucloxacillin sodium and colistimethate sodium, further discussion was ongoing in group 7
  - whether trials should be started for introducing MAT into the remaining monographs,
  - whether group 7 should wait for sufficient validation data from other manufacturers,
  - or whether a general statement should be included into individual monographs
- Some members indicated difficulties developing a MAT for certain substances
- Waiting for further validation data seems not to be promising since more than 10 years have passed already
- A general statement to be included into individual monographs was considered and discussed internally with members of the BET WP

MAT or BET for control of pyrogens in antibiotics?

- The following general statement was considered:
  "**Pyrogens:**
  If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of pyrogens, the presence of relevant amounts of pyrogens is ruled out by a product-specifically validated MAT (2.6.30) taking into account the limits given in Ph. Eur. 5.1.10. If justified by a product-specific risk assessment, routine testing using BET (2.6.14) may be sufficient if approved by the competent authority."
- However, group 7 did not reach a conclusion on that matter but it seems that these considerations regarding a general statement instead of a concrete test in an individual monograph led to a more general discussion within the EDQM how to remove the RPT completely from the Ph. Eur. – and here we are now

MAT or BET for control of pyrogens in antibiotics?

BET (2.6.14 and 2.6.32)	MAT (2.6.30)
Test interferences are seldom for antibiotics, usually solvable by dilution of test solution and controlled by positive product control	Detects all pyrogens in samples
Complexing agents and twitter ions as potential interferences from the finished product are seldom used in antibiotics	Test principle is concordant with situation in humans
Reagents and kits commercially available	Kits commercially available
Very sensitive test	Less sensitive test; sensitivity for endotoxins and NEPs is different
NEPs are not detected (only ß-glucans with 2.6.14 but less sensitive; not at all with 2.6.32)	Development and validation of test necessary for concrete product

MAT or BET for control of pyrogens in antibiotics?

- However, the main question is: is the MAT suitable for antibiotics?

PRODUCT EVALUATION TEST REPORT

7. CONCLUSIONS

The pyrogen test on rabbits shows no obvious abnormalities and no abnormal temperature responses demonstrating that [redacted] did not produce fever and so pyrogenic reaction in rabbits.

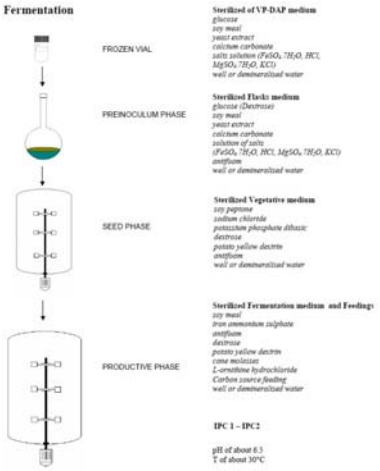
Based on the obtained results of the MAT assay, the testing shows it is not suitable for tested products. For each tested batch (S1, S2 and S3) in the neat, 1:9 and 1:18 dilution we observe masking effect of endotoxin-spike (0.5 EU/mL) which reflects on the %recovery results < 50%. In addition for sample 3 (S3) masking of endotoxin was observed for dilution 1:36. Therefore, the MAT assay is not suitable to test the Active Product Ingredients (API) of Flucloxacillin drug product. Note: MVD: 1 : 37.6

➔ It seems that MAT may not be suitable for antibiotics; at least not for all

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

- Current situation for monographs on antibiotics still having the RPT:
  - Validation data not sufficient to switch in general to BET
  - MAT most likely not suitable at least for flucloxacillin sodium and colistimethate sodium
  - RPT not yet removed; however, use is clearly discouraged
- ➔ Risk assessment on process level seems to be the only suitable solution
- Risk assessment to be performed by the active substance manufacturer being the only one who has the necessary knowledge of the actual manufacturing process
- Introduction of Ph. Eur. 5.1.13 and removal of all RPTs from monographs as currently proposed in Pharmeuropa 35.1 supports this approach

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?



Example for a typical fermentation process for an antibiotic

- The producing microorganisms is taken from the WCB (working cell bank) and the number of cells is increased step by step by feeding with different culture media
- The composition of the culture media depends on the actually used microorganisms and the target compound
- Use of additional amino acids beyond the common C and N sources is quite common dependent on the biosynthetic pathway for the target compound

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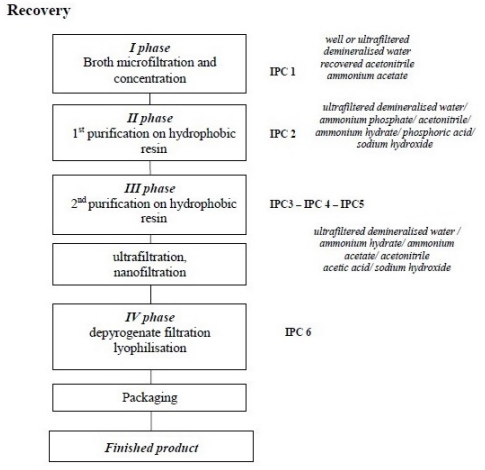
Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?



Example for a typical recovery process for an antibiotic manufactured by fermentation

- Cell debris is removed by filtration
- Additional purification using hydrophobic or ion exchange resins is quite common
- Ultrafiltration and nanofiltration is common, too
- Lyophilisation is common as most antibiotics are susceptible to hydrolysis

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Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

Example for typical filtration details for an antibiotic manufactured by fermentation

- Nanofiltration and ultrafiltration can be used to further separate the target compound from substances of lower and higher molecular weight
- Most antibiotics have molecular weights between 300 and 2000 Da

Step of the manufacturing process	*Type / Material*	*cut-off*	*Brand*
Recovery: microfiltration -diafiltration	ceramic membranes	About 0.1 µm	Pall or alternative
Recovery: nanofiltration	Spiral wound membranes in polyamide	About 150-300 Dalton	Koch/Suez/HR or alternative
Recovery: ultrafiltration	Membranes Polysulfone Hollow Fiber	About 10 000 Dalton	Microza/Asahi or alternative
Recovery: final nanofiltration	Spiral wound membranes in polyamide - dimension 4040 and 8040	About 150-300 Dalton	Koch/Suez/HR or alternative
Recovery: depyrogenate filtration	Cartrige Filter N66 disposable	About 0.2 µm	Pall or alternative
Step of the manufacturing process	*Type / Material*	*Pore size*	*Brand*
Recovery: purification	HP20SS Polystyrene/divinylbenzene matrix Resin	About 60 – 150 µm	Mitsubishi chemical

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

What should be taken into account for a risk assessment for an antibiotic manufactured by fermentation?

- Properties of the strains for the production process (prokaryotic or eukaryotic; if prokaryotic, whether gram+ or gram- and the corresponding potential for presence of pyrogens like lipoteichoic acid (LTA) or peptidoglycans; potential expression of certain components (e.g. exotoxins or other proteins); etc.)
- Components of the media and their origin (e.g. animal-derived (-> virus?), plant-derived (-> mycotoxins?), or synthetic)
- Bioburden of raw materials including type of bioburden (i.e. g+, g-, fungi) (relevant for C and N sources; salts; solvents; other additives; water)
- Potential cross contamination within the manufacturing process as well as environmental monitoring

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

What should be taken into account for a risk assessment for an antibiotic manufactured by fermentation?

- Capability of the downstream process to remove pyrogens (examples)
  - Heat sterilisation of culture media to inactivate heat-labile pyrogens
  - Extraction processes using organic solvents that dissolve the target compound but not water-soluble pyrogens (if applicable)
  - Filtration processes to separate substances with higher molecular weight like proteins or DNA/RNA
  - pH variations by using strong acids or alkali that may destroy certain pyrogens (if applicable; i.e. target compound is stable)
- Verification of the assessment by spiking experiments if MAT is not possible (not easy to develop but needed to conclude on the role of NEPs)

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

What should be taken into account for a risk assessment for (semi-)synthetic substances?

- Microbial contamination (-> bioburden) of raw materials; type of bioburden (i.e. gram+; gram-; fungi) may need further evaluation
- Holding times with process conditions allowing growth of microorganisms potentially present
- More detailed considerations are necessary when a raw material is a fermentation product (see before)
- Potential cross contamination within the manufacturing process as well as environmental monitoring
- Potential of the downstream process (purification) to remove NEPs
- Verification of the assessment by spiking experiments if MAT is not possible

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

Type of pyrogenic substance	Primary source	Likelihood of presence	Possible control mechanisms for risk mitigation
Endotoxin associated proteins	Water	Medium	• WFI: bioburden + endotoxins • Ultrafiltration for separation • Depyrogenation of vials; stoppers washed + autoclaved
Enterotoxins	Raw materials, skin bacteria	Medium	• Bioburden of raw materials • More common with *Staphylococci* + *Streptococci*
Lipoarabinomannans (from mycobacteria)	Clinical specimens	Low (if not routinely isolated)	• Most potent: *Mycobacterium tuberculosis*; presence in process area not likely • Monitoring of staff might be required in certain countries
DNA/RNA	Cells	Low	• Removal of cell debris

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Source: Sandle, Tim. (2015). Assessing Non-endotoxin Microbial Pyrogens in Relation in Pharmaceutical Processing. Journal of GXP Compliance. 19. 1-12; modified

Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

Type of pyrogenic substance	Primary source	Likelihood of presence	Possible control mechanisms for risk mitigation
Fungal components (e.g. mannan; glucan; mannoprotein)	Raw materials; Air conditioning	Low (if not routinely isolated)	• Environmental monitoring • ß-glucans can be detected with BET (but very low sensitivity)
Parasite components (e.g. phosphoinositol)	Insects; food; people	Low	• Control of parasites necessary (e.g. insect grilles)
Solid materials (e.g. plastic disposables)	Process components	Medium	• Plastic used for processing come certified as „pyrogen-free“ • Qualified for Extractables and Leachables
Drugs (e.g. steroids; bile salts; cytokines)	API; raw materials	Low	• Control of raw materials if used • Control of cross contamination
Plant alkaloids	Plants	Low	• Control of raw materials if used

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Risk assessment – how likely is the presence of Non-Endotoxin-Pyrogens (NEPs) in antibiotics?

Type of pyrogenic substance	Primary source	Likelihood of presence	Possible control mechanisms for risk mitigation
Peptidoglycans, Muramylpeptides, Porins (from cell wall)	Raw materials, skin bacteria	Medium	• Bioburden of raw materials
LTA and other gram+ bacterial cell wall components	Raw materials; skin bacteria	Medium	• Bioburden of raw materials and at key process steps
Exotoxins	Raw materials; skin bacteria	Medium	• Bioburden of raw materials and at key process steps
Antitumor agents	Chemicals	Low	• Control of raw materials if used
Viruses	Animal raw materials	Medium	• Viral inactivation steps (solvent-detergent; nanofiltration; heat)

Expectations from a regulatory point of view

Preliminary Remark

- No retrospective assessment of pyrogenicity is necessary (see briefing note in the draft monograph 5.1.13 in Pharmeuropa 35.1 (PA/PH/Exp. BET/T (21) 13 ANP))
- Nevertheless, the Marketing Authorisation Holder (MAH) is ultimately responsible for his medicinal product

Expectations from a regulatory point of view

Impact of changes in the general monograph "Parenteral Preparations"

- Current monograph states that parenteral preparations for human use comply with the test for bacterial endotoxins (2.6.14; LAL) or where justified and authorised with the test for pyrogens (2.6.8; RPT)
- Proposed monograph states that parenteral preparations for human use comply with a suitable test for pyrogenicity – Guidance for the selection of a test is given in general chapter 5.1.13
- **Ph. Eur. 5.1.13** indicates under the heading "choice of test" that the test for **bacterial endotoxins (2.6.14 or 2.6.32) is appropriate only if the presence of non-endotoxin pyrogenic substances can be ruled out**
- Risk assessment on potential presence of NEPs is mandatory if BET is chosen as sole method for testing pyrogenicity of a given product
- MAT is to be used if presence of NEPs cannot be ruled out

Expectations from a regulatory point of view

Already authorised approaches for control on pyrogens are not questioned

- If a medicinal product or API is authorised to be controlled by means of BET only, then no further action is necessary according to the briefing notes in Pharmeuropa 35.1 for the affected monographs
- However, the MAH is ultimately responsible for the quality of an authorised medicinal product
- There was a case about 12 years ago with one gentamicin batch complying with the BET but not with the RPT; the reason for non-compliance with the RPT was not found
- Thus, a risk evaluation on potential presence of NEPs is recommended even for already authorised processes
- However, in line with the briefing note, a risk assessment will not be requested by a competent authority unless an issue with NEPs becomes known

Expectations from a regulatory point of view

Expectations for a parenterally administered medicinal product where control on pyrogens is made by means of BET only

- Situation:
  Medicinal product is authorised but relevant changes at the manufacturing process potentially influencing the presence of NEPs are proposed (e.g. different raw materials, production sites, or process parameters):
  - Risk assessment on potential presence of NEPs in the medicinal product after the change is mandatory according to Ph. Eur. 5.1.13 and should be provided with the variation application
  - Verification of risk assessment using MAT can be requested in accordance with Ph. Eur. 5.1.13 "*if any changes are made to the production process that could influence the quality of the product with regard to pyrogenicity, it is recommended to repeat the monocyte-activation test*"

Expectations from a regulatory point of view

Expectations for a parenterally administered medicinal product where control on pyrogens will be made by means of BET only

- Situation:
  Application for a new medicinal product:
  - Risk assessment on potential presence of NEPs in the applied medicinal product is mandatory according to Ph. Eur. 5.1.13 and should be provided with the marketing authorisation application if a completely new source of API and/or excipient is used that has never been authorised in the EU/EEA before (or if NEPs may be introduced during the manufacturing process of the medicinal product)
  - Verification of risk assessment using MAT can be requested (see Ph. Eur. 5.1.13)
  - If a known source (already authorised somewhere in the EU/EEA) is to be used, a risk assessment is formally not necessary (but recommended)

Conclusion

1. MAT

The MAT is the only test considered to detect all relevant pyrogens that may be harmful for humans when the RPT is phased out.

2. BET

The BET (LAL; recombinant factor C) detects bacterial endotoxins only. While these are considered as the most powerful and relevant pyrogens, they are not the only ones to be taken into account.

3. Potential Presence of Non-Endotoxin Pyrogens

Whenever the BET is the only test used for pyrogene testing, potential presence of non-endotoxin pyrogens needs to be ruled out by means of a sound risk assessment, if applicable, sufficiently verified by the MAT.

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Thank you very much for your attention! Any questions?

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Federal Institute for Drugs and Medical Devices

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMAs regulatory science strategy in practice - Regulatory acceptance of 3R testing approaches

EDQM-EPAA Pyrogenicity Event

Presented by Beken Sonja on 15 February 2023
3Rs Working Party (EMA)

An agency of the European Union

Animal use in the EU

EUROPEAN MEDICINES AGENCY

10,4 million animals used in 28 Member States incl Norway (2019)
Publicly accessible version of the ALURES Statistical EU Database on animal use
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm

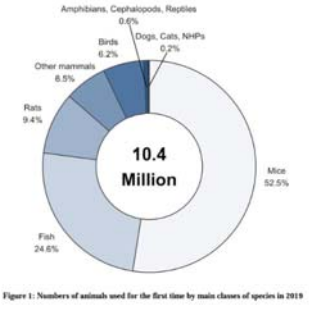

Figure 1: Numbers of animals used for the first time by main classes of species in 2019

Regulatory use:

	Quality control (incl batch safety and potency testing)	Toxicity and other safety testing including pharmacology	Other efficacy and tolerance testing
Legislation on medicinal products for human use	715,652	313,983	64,195
Legislation on medicinal products for veterinary use and their residues	240,853	43,552	31,960
Medical devices legislation	2,646	49,735	1,332
Industrial chemicals legislation	0	153,940	457
Plant protection product legislation	180	68,036	647
Biocides legislation	0	1,905	552
Food legislation including food contact material	168	36,520	30
Feed legislation including legislation for the safety of target animals, workers and environment	19	7,092	9,351
Other legislation	694	45,092	188
Total	960,212	719,855	108,712

HMPs

Regulatory uses: Quality control	Number of uses	Percentage
Pyrogenicity testing	28763	4.02%
Batch safety testing	97318	13.60%
Batch potency testing	563989	78.81%
Other quality controls	25582	3.57%
Total	**715652**	**100,00%**

VMPs

Regulatory uses: Quality control	Number of uses	Percentage
Batch potency testing	180657	75.01%
Batch safety testing	53371	22.16%
Other quality controls	6684	2.78%
Pyrogenicity testing	141	0.06%
Total	**240853**	**100,00%**

Classified as internal/st

Directive 2010/63/EU of the EP and of the Council

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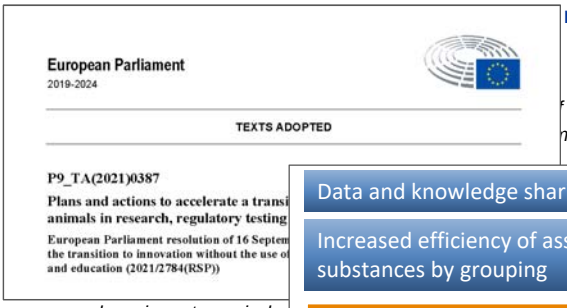
mals used for scientific purposes



European Parliament
2019-2024

TEXTS ADOPTED

P9_TA(2021)0387

Plans and actions to accelerate a transi
animals in research, regulatory testing

European Parliament resolution of 16 Septem
the transition to innovation without the use o
and education (2021/2784(RSP))

scientifically satisfactory method or testing strategy, not
f a procedure .
nals used in projects is reduced to a minimum without

procedure is not carried o
the use of a live animal, is

2. In choosing between pro
selected:

(a) use the minimum nu
(b) involve animals with
(c) cause the least pain,
and are most likely to p

Data and knowledge sharing: PARERE and other mechanisms

10/02/2022

Increased efficiency of assessing substances by grouping

One substance – One assessment, see 'ONE – Health, Environment, Society - Conference', June 2022 Brussels

3Rs in R&D of medicines EMA and 3Rs

ALURES statistical database and open-access database on non-technical summaries of authorised projects

IMI and H2020/Horizon Europe and European Research Council

EURL-ECVAM reviews on NAMs in biomedical research

Training programmes on 3Rs

EPAA as means for collaboration

https://oeil.secure.europarl.europa.eu/oeil/popups/ficheprocedure.do?reference=2021/2784(RSP)&l=en&mc_cid=687873d92e&mc_eid=dba5dcb0dc

EMA's Regulatory Science Strategy

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Driving collaborative evidence generation – improving the scientific quality of evaluations

Core recommendations	*Underlying actions*
Leverage non-clinical models and 3Rs principles	‣ Stimulate developers to use novel pre-clinical models where appropriate, including those adhering to the 3Rs: » Cooperate with other EU agencies/bodies to fund research and (access to) standardised repositories for alternative methods and models; » Development of clear guidance to encourage and prioritise the use of New Approach Methodologies (NAMs) that can be used to fulfil testing requirements in lieu of traditional animal tests and that take the 3Rs into serious consideration; ‣ Re-focus the role of the Joint 3Rs working group (J3R WG) to support qualification of new alternative 3R-compliant methods/models including in silico and novel in vitro assays; ‣ Implement/develop IT tools to exploit the added value of SEND for the re-analyses of non-clinical studies to support clinical trials, marketing authorisation and improved evidence generation.

- engagement with stakeholders to create communications channels and establish a good European regulatory network on NAMs

https://www.ema.europa.eu/en/about-us/how-we-work/regulatory-science-strategy#regulatory-science-strategy-to-2025-section

- core recommendations dedicated to leverage and qualification of 3Rs methods
- raise awareness and start discussing/defining context of use, endpoints and reference compounds

Reinforce and further embed application of the 3Rs principles	‣ Apply the highest possible 3Rs standards when implementing the Veterinary Medicines Regulation (EU) 2019/6 as well as other legislative documents/guidelines to stimulate developers to use novel approaches adhering to 3Rs standards; ‣ Strengthen cooperation between all stakeholders and international partners: » Cooperate with other EU agencies/bodies to fund research and (access to) standardised repositories for alternative methods and models; ‣ Promote in silico methodology (e.g. modelling), novel in vitro assays and systematic reviews to reduce animal use, particularly in toxicology/ epidemiology and batch control: » Re-focus the role of the Joint 3Rs working group (J3R WG) to support qualification of new alternative 3R-compliant method/models including in silico and novel in vitro assays; » Development of clear guidance to encourage and prioritise the use of NAMs that can be used to fulfil testing requirements in lieu of traditional animal tests and that take the 3Rs into serious consideration; ‣ Promote regulatory acceptance and training.

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EMA´s comittment to the 3Rs

https://www.ema.europa.eu/en/human-regulatory/research-development/ethical-use-animals-medicine-testing

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

23 September 2011
EMA/470807/2011
Veterinary Medicines and Product Data Management

Statement of the EMA position on the application of the 3Rs (replacement, reduction and refinement) in the regulatory testing of human and veterinary medicinal products

The European Medicines Agency (EMA) commits to the application of replacement, reduction and refinement (the 3Rs) of animal testing as detailed in Directive 2010/63/EU[1]. To this end, a Joint ad hoc Expert Group (the JEG 3Rs) has been created in order to promote best practice in the implementation of the 3Rs in regulatory testing of medicinal products and to facilitate full and active cooperation with other European groups working in the 3Rs area.

While significant progress has been made in relation to regulatory testing involving animals it remains the case that certain types of data can only be generated by means of animal studies. Where such studies are needed they should be selected and conducted in strict adherence to the 3Rs principles.

As a European body with responsibility for developing harmonised European regulatory requirements for human and veterinary medicinal products the EMA has and will continue to play a key role in eliminating repetitious and unnecessary animal testing in the European Economic Area (EEA), in collaboration with other European organisations such as EDQM. Through its active participation and collaboration in the work of other multinational organisations such as the ICH and the VICH, the EMA contributes to the application of the 3Rs in the development of globally harmonised requirements, the implementation of which contributes to the elimination of unnecessary animal testing.

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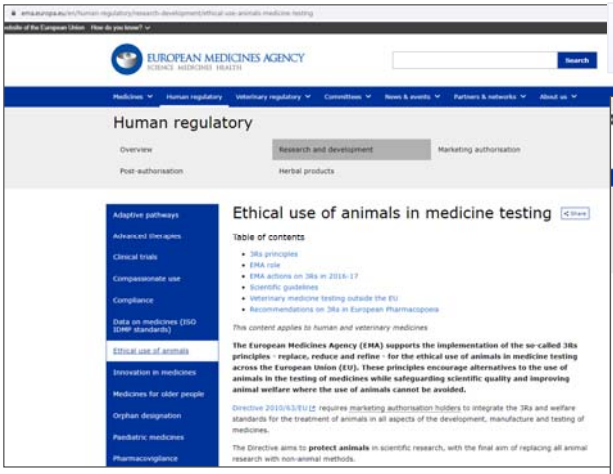
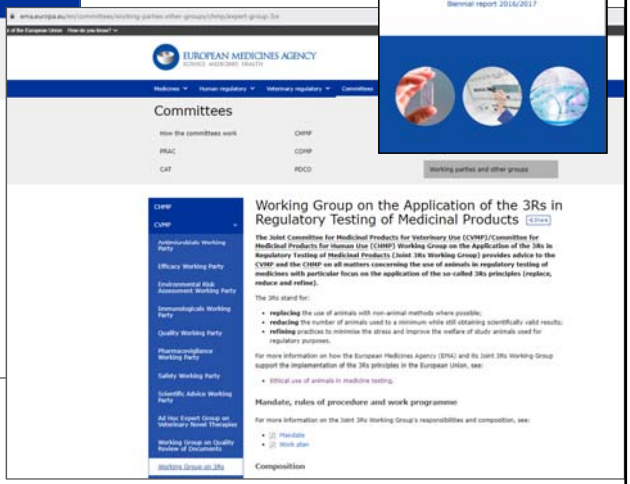
EMA and the 3Rs



JEG3Rs and J3RsWG
2010 -2016



https://www.ema.europa.eu/en/human-regulatory/research-development/ethical-use-animals-medicine-testing

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Guideline on regulatory acceptance of 3Rs

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Regulatory acceptance :

- Incorporation of a new 3R testing approach into a regulatory testing guideline
- On a case-by-case basis: acceptance by regulatory authorities of new approaches not (yet) incorporated in testing guidelines but used for regulatory decision making

Criteria for regulatory acceptance

- Defined test methodology (protocol, endpoints)
- Relevance within a particular context of use (including accuracy)
- Context of use (including limitations).
- Reliability/robustness
- Voluntary submission of data obtained by using a new 3Rs testing approach can be made in parallel with data generated using existing methods (safe harbour)


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Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches

https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-principles-regulatory-acceptance-3rs-replacement-reduction-refinement-testing-approaches_en.pdf

Procedure

Guideline on Qualification of Novel Methodologies for Drug Development (EMA/CHMP/SAWP/72894/2008 Rev. 1)

https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-advice-protocol-assistance/qualification-novel-methodologies-medicine-development-0#chmp-qualification-opinions-section

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Past activities

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- Review of final product batch testing requirements (centralised authorized products) – continuous collaborative effort with IWP, BWP, VWP and QWP. Product specific recommendations are made directly to MAHs with endorsement from either CHMP or CVMP.
- Reflection papers providing an overview of the current regulatory testing requirements for medicinal products for human and veterinary use and opportunities for implementation of the 3Rs (EMA/CHMP/CVMP/3Rs/742466/2015 & EMA/CHMP/CVMP/3Rs/164002/2016)
- Recommendation to MAHs, highlighting the need to ensure compliance with 3Rs methods described in the European Pharmacopoeia (EMA/CHMP/CVMP/JEG-3Rs/252137/2012, HMPs & VMPs)
- Recommendation to MAHs, highlighting recent measures in the human/veterinary field to promote reduction, refinement and replacement (3Rs) measures described in the European Pharmacopoeia (EMA/CHMP/CVMP/3Rs/336802/2017 VMPs from 01/01/2017, EMA/CHMP/CVMP/3Rs/614768/2017 HMPs from 01/01/2018)
- Guidance for individual laboratories for transfer of quality control methods validated in collaborative trials with a view to implementing 3Rs (EMA/CHMP/CVMP/JEG- 3Rs/94436/2014)
- Supporting CVMP input into VICH GL50 & GL55, waiver TABST, new draft LABST
- Report on actions taken in the review and update of EMA guidelines to implement best practice with regard to 3Rs in regulatory testing of medicinal products (EMA/CHMP/CVMP/JEG-3Rs/677407/2015)
- CVMP position statement on the ethical use of animals in the development, manufacture and testing of veterinary medicines (EMA/CVMP/3Rs/506841/2017)
- Collaboration with EC, EDQM, other EU agencies and international organisations an projects (e.g. Vac2Vac)

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Regulatory testing requirements & opportunities for 3Rs implementation (2018)

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Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Pyrogens (Rabbits)* *test also applicable to biological products	European Pharmacopoeia (Ph.Eur.) 2.6.8	Amikacin-sulfate, Calcium levulinate dihydrate, Colistimethate sodium, Chloramphenicol sodium succinate, Dicloxacillin sodium, Flucloxacillin sodium, Glucose, Glucose monohydrate, Kanamycin acid sulphate, Kanamycin monosulfate, Polymyxin B sulphate, Sodium citrate. Besides the active substances in the table, the test is used in case of derived medicinal products and some older products.	According to specific Ph. Eur. monographs, this test should be used if the active substance is intended for use in the manufacture of parenteral preparations without further appropriate procedure for the removal of pyrogens. Therefore, in practice, the pyrogen test is seldom performed on the active substances. In addition, the latest version of Chapter 2.6.8 (published in edition 9) includes the following text: 'In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. Wherever possible and after product specific validation, the pyrogen test is replaced by the monocyte-activation test (2.6.30).'	To communicate that the test shall be used only in the justified and authorised cases when neither the Monocyte-activation test (MAT, 2.6.30, Ph.Eur.) nor the Bacterial Endotoxins test (BET, 2.6.14, Ph.Eur.) can be performed (see Ph.Eur. general monograph Substances for pharmaceutical use and Chapter 2.6.30). For new applications for marketing authorisation of medicinal products, the MAT and BET should be considered as the first choice for validation and submission. In the case of older products the pyrogen test should be replaced after demonstration of suitability of MAT or BET for the product via variation procedures.

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Regulatory testing requirements & opportunities for 3Rs implementation (2018)

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Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
			monocyte-activation test (2.6.30).	
Bacterial Endotoxins (amoebocyte lysate from *Limulus polyphemus* or *Tachypleus tridentatus*)* *test also applicable to biological products	Ph. Eur. Chapter 2.6.14.	Active substances of endotoxin-free grade and most of medicinal products intended for parenteral administration.	Often used as an alternative to the pyrogen test. The BET is used to detect or quantify endotoxins from Gram-negative bacteria using Limulus Amoebocyte Lysate obtained from blood cells (amoebocytes) of horseshoe crabs (*Limulus polyphemus, Tachypleus tridentatus*).	BET assays based on recombinant Factor C, a non-animal derived reagent, are available. Their use is referred to in Ph.Eur. chapter 5.1.10, "Guidelines for Using the Test for Bacterial Endotoxins", Section 12.2 states: *The use of alternative reagents such as recombinant factor C as a replacement to the amoebocyte lysate eliminates the use of a reagent extracted from live animals. Replacement of a rabbit pyrogen test or a bacterial endotoxin test prescribed in a monograph by a test using recombinant factor C reagent or any other reagent as a replacement of the amoebocyte lysate is to be regarded as the use of an alternative method in the replacement of a pharmacopoeial test, as described in the General Notices.*

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Specific 3Rs recommendations - PhEUR

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SCIENCE MEDICINES HEALTH

12 July 2012
EMA/CHMP/CVMP/3EG-3Rs/252137/2012
Committee for Medicinal Products for Human Use (CHMP)
Committee for Medicinal Products for Veterinary Use (CVMP)

Recommendation to marketing authorisation holders, highlighting the need to ensure compliance with 3Rs methods described in the European Pharmacopoeia
Applicable to all medicinal products regardless of type

Therefore, in order to comply with the provisions of Directive 2010/63/EU and to secure an undisrupted supply of medicinal products to the European Market, MAHs should take all necessary actions to introduce 3Rs Ph. Eur. methods including submission of variations to marketing authorisations as appropriate.

Document	Applies to
Recommendation highlighting the need to ensure compliance with 3R methods described in the European Pharmacopoeia	All medicinal products
Recommendation highlighting recent measures in the human field to promote 3Rs measures described in the European Pharmacopoeia	Human vaccines
Recommendation highlighting recent updates for the 3Rs methods described in the European Pharmacopoeia applicable to human vaccines against hepatitis A	Human vaccines against hepatitis A
Recommendation highlighting recent measures in the veterinary field to promote 3Rs measures described in the European Pharmacopoeia	Veterinary vaccines
Recommendation for veterinary vaccines, highlighting the need to update marketing authorisations to remove the target animal batch safety test (TABST) following removal of the requirement from the European Pharmacopoeia monographs	Veterinary vaccines

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The new 3RsWP

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- Composition

Sonja Beken (Chair)	BE	FAGG-AFMPS-FAMHP	Human MPs - NCWP, Non-Clinical
Sarah Adler-Flindt (Vice-Chair)	DE	Federal Office of Consumer Protection and Food Safety	Veterinary MPs - Non-Clinical
Elisabeth Balks	DE	PEI	Veterinary MPs - Batch release
Kathrine Just Andersen	DK	Danish Medicines Agency	Veterinary MPs - EWP-V, Non-Clinical and Clinical
Camilla Svensson	SE	MPA	Human MPs - Non-Clinical
Peter Theunissen	NL	MEB	Human MPs - Non-Clinical

- EMA support to 3RsWP
  - Scientific secretariat: Stefano Ponzano (H-Division), Michael Empl (Vet-division)
  - Administrative secretariat: Stavroula Tasiopoulou (H-division)
- 3RsWP Web Page

  https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/3rs-working-party
- First stakeholder meeting scheduled for 28th of February 2023

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An ambitious 3Rs workplan with a vision to the future

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High level strategic goals:

- Assume a **strategic role in the field of the 3Rs** with strengthened **cooperation** between all stakeholders and international partners
- Move non-clinical assessment from discovery toxicology towards regulatory use and acceptance of animal-free innovations or new approach methodologies (NAMs) (for hazard identification, toxicity prediction, ADME modelling, disease modelling)
- Ensure **follow-up of the 3Rs in batch release testing of human and veterinary medicinal products**
- **Review and update of EMA guidelines** to implement **best practice regarding 3Rs** and **impact monitoring** of implemented changes (including identification of new actions)
- Follow up of actions following EP resolution of 16 September 2021 on plans and actions to accelerate the transition to innovation without the use of animals (2021/2784(RSP))
- Follow-up and identification of actions related to alternatives to the use of non-human primates

https://www.ema.europa.eu/documents/other/non-clinical-working-party-consolidated-three-year-work-plan-non-clinical-domain_en.pdf

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3RsWP – specific workplan actions

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- **Review** of **product batch testing requirements** with regards to the application of the 3Rs (human and veterinary)
- Perform a **review** of the **most promising available 3Rs methodologies** that could be considered for qualification, i.e. identify animal tests where the largest impact from a move to alternative/non-animal testing would apply
- **Collaboration** with the Methodology domain with respect to **modelling and simulation**, to support the regulatory acceptance of NAMs
- Establish an easily accessible **database for qualified/validated NAMs** together with e.g. EDQM and EURL-ECVAM
- Organise **annual multistakeholder 3RsWP brainstorming sessions** on emerging 3Rs topics
- Organise an **EMA 3RsWP-led multistakeholder conference** to showcase the achieved progress with regards to 3Rs in the field of human and veterinary medicinal products and to introduce the new 3RsWP and future workstreams
- Develop **training** activities on 3Rs methods and best 3Rs practices across the EU network.

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3RsWP – workplan actions & global harmonisation

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Creation of a **worldwide cluster of regulators** to establish **regulatory acceptance criteria for NAMs** and to **harmonise views and regulatory acceptance criteria between the EU and worldwide regulators**

Qualification of NAMs

Development of COU-based qualification criteria

- **ICH S5R3** related activities: **support qualification of EFD in vitro/ex vivo/other 3Rs approaches** and follow up of 3Rs impact.
- **Q&A ICH S7B** related activities: support qualification of in **vitro/ex vivo/other 3Rs approaches** and follow up of 3Rs impact.
- Support to **the Innovation Task Force and Scientific Advice Procedure** for **Regulatory acceptance and Qualification Advice/Opinion** for NAMs
- Review of **skin sensitization testing** recommendations by OECD in the light of applicability for topically applied medicinal products
- Reflection paper to **define regulatory acceptance criteria for organ-on-chip technologies** for **specific contexts of use**
- Follow-up **workshops on MPS** with a specific focus towards method **qualification** for regulatory acceptance.

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Collaboration with EMA's Innovation Task Force on 3Rs

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Multidisciplinary: scientific, regulatory & legal

Dedicated forum for early dialogue between regulators and stakeholders (e.g. SMEs, academics, researchers, research and public-private funded consortia (e.g. IMI), pharmaceutical industry)

Focus on emerging therapies, methodologies & technologies

NEW focus on regulatory acceptance of so-called new approach methodologies (NAMs) to replace the use of animals in the testing of medicines (3Rs)

→ e.g., *in silico* modelling & novel *in vitro* assays (e.g. MPS technology)

Objectives are to encourage the development of NAMs and accelerate their integration in the regulatory framework for the development and evaluation of medicines

Informal exchange of information and provision of guidance (non-legally binding) **early** in the development process during briefing meetings

Discussion led by multidisciplinary experts from the Agency network, and EMA working parties & committees **– best available scientific expertise**

The briefing meetings are **free of charge**

https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines#ema's-innovation-task-force-(itf)-section

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Take home messages

The European Regulatory Network is open to 3Rs

The new 3RsWP is the official 3Rs hub at the EMA

Recommendations from 3RsWP and specific follow-up actions to promote 3Rs measures described in the European Pharmacopoeia will be undertaken

Flexibility regarding guideline requirements:

- impact on Reduction and Refinement of animal use
- based upon scientific rationale
- scientific advice (EMA Scientific Advice Working Party)

Qualification/validation of novel 3R testing approaches *(in vitro, in silico, ex vivo, …)*:

- (extent of) qualification criteria to be defined in line with context of use
- early dialogue with regulatory authorities is encouraged
- Collaboration is key to achieve progress towards regulatory acceptance of 3Rs methods

Close collaboration with ITF 3Rs: essential tool for early engagement and feedback

The set-up of an informal cluster of regulators is considered instrumental to foster global early collaboration on 3Rs

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Any questions? Suggestions?

Further information

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Telephone +31 (0)88 781 6000
Send us a question Go to www.ema.europa.eu/contact

Follow us on **@EMA_News**

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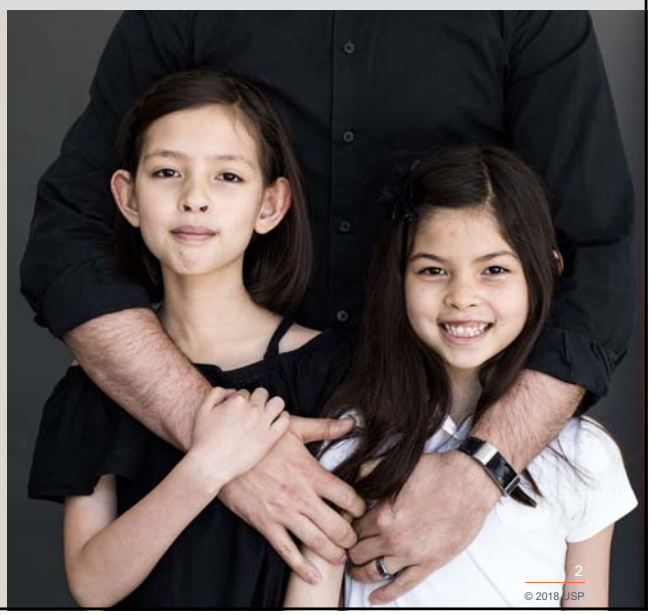
Pyrogen Testing in the US: Past, Present, and Future

Leslie Furr, MS

February 2023

Agenda

- USP Overview
- Focus on 3 R's
- History and background
- A look into the future



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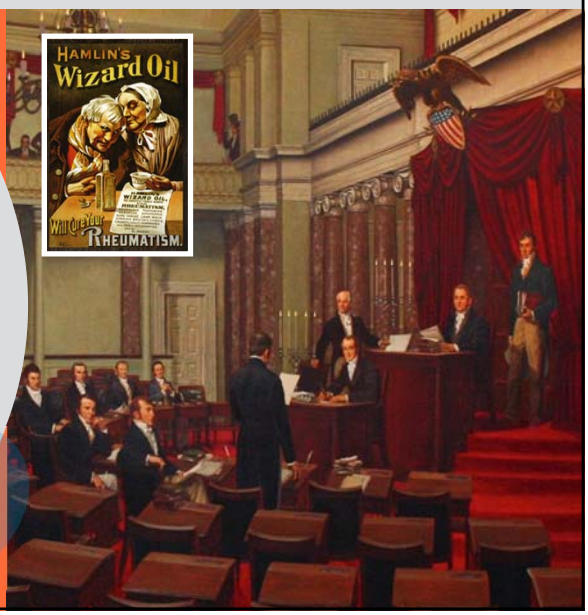
USP Overview

Our enduring mission

To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.



For quality standards to be impactful, they must be…

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Ensuring standards have impact

- To date, our standards impact 2 billion people globally – but our commitment to empower a healthier tomorrow doesn't stop there.
- As medicines come to market and public health issues emerge, new standards must be created to address public need.
- Standards evolve to keep pace with industry changes and to respond to public health challenges.

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Collaborative Groups

Biologics	Small Molecules	Excipients	General Chapters	Healthcare Quality & Safety	Dietary Supplements & Herbal Medicines, Food Ingredients
Biologics Monographs 1 - Peptides & Oligonucleotides *Michael De Felippis*	**Small Molecules 1** *Mary Seibel*	**Simple Excipients** *Eric Munson*	**General Chapters-Dosage Forms** *Martin Coffey*	**Nomenclature & Labeling** *Stephanie Crawford*	**Botanical Dietary Supplements & Herbal Medicines** *Robin Marles*
Biologics Monographs 2 - Proteins *Wendy Saffell-Clemmer*	**Small Molecules 2** *Justin Pennington*	**Complex Excipients** *Otilia Koo*	**General Chapters - Chemical Analysis** *Nancy Lewen*	**Healthcare Safety & Quality** *Melody Ryan*	**Non-botanical Dietary Supplements** *Guido F Pauli*
Biologics Monographs 3 - Complex Biologics & Vaccines *Earl Zablackis*	**Small Molecules 3** *Eric Kesslen*	**Excipients Test Methods** *Chris Moreton*	**General Chapters-Microbiology** *Mark Schweitzer*	**Compounding** *Brenda Jensen*	**Dietary Supplements Admission Evaluation & Labeling** *Tieraona Low Dog*
Biologics Monographs 4 - Antibiotics *Matthew Borer*	**Small Molecules 4** *Kim Huynh-Ba*		**General Chapters-Packaging & Distribution** *Renaud Janssen*	**Healthcare Information & Technology** *Jeanne Tuttle*	**Food Ingredients** *Jon DeVries*
Biologics Monographs 5 - Advanced Therapies *Mehrshid Alai*	**Small Molecules 5** *Amy Karren*		**General Chapters - Measurement & Data Quality** *Jane Weitzel*		
	Over-the-Counter (OTC) Methods & Approaches *Raphael Ornaf*		**General Chapters-Statistics** *Charles Tan*		
			General Chapters - Physical Analysis *Richard Meury (pro tempore)*		

General Chapter Creation/Revision Process

Posting on the Pharmacopeial Forum

1. Revision is Determined

Through every 5-year cycle, each General Chapter is reviewed and considered part of the EC workplan.

Revisions may also be carried over from the previous cycle, as indicated on the Legacy document.

Revisions can also be requested from internal staff or external stakeholders.

2. External Stakeholder Communications

Depending on the potential scope of the revision, the EC/EP/SC may choose to communicate their intent to revise through various USP publications, including Notice of Intent to Revise, General Chapter Prospectus, or even a Stimuli to the Revision Process Article.

3. Proposal is Drafted by EC/EP/SC

In process revisions are typically drafted through Working Groups.

Often, a Working Group will divide and conquer the revisions, and review the revisions in periodic meetings.

Literature research and Expert Advisors may be needed to further develop a revision.

4. Proposal Submitted to Publications

The EC/EP/SC will target a PF version to submit the proposed revision.

Deadlines are established by the Publications department in order to ensure every step in the process is completed.

The Publications process includes GC dependency, developmental review (editorial, tagging, USP style), production (online publication).

5. Public Comment Solicited through PF

Standard Revision Process calls for publication in the *PF* for a 90-day notice and comment period.

Any external stakeholder can submit a comment through the PF online platform.

Once the comment is received, the SL is responsible for consolidating them and disseminating them to the EC/EP/SC.

6. Comments Reviewed by the EC/EP/SC

The EC/EP/SC convene to review the comments and discuss whether to incorporate, partially incorporate, or not incorporate comments into the proposal.

In many cases, the comments received are substantial enough that the PF process is repeated from Steps 3 - 6.

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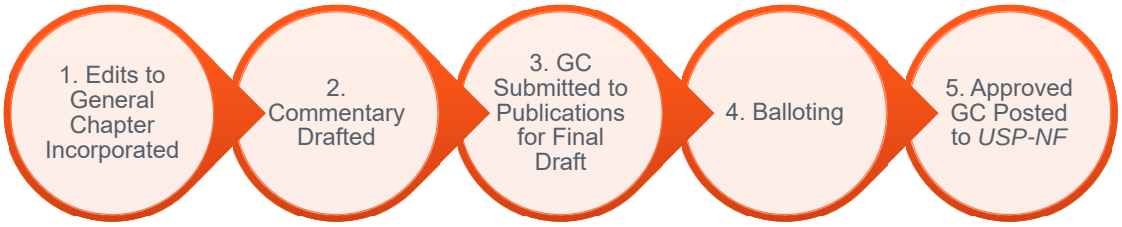
General Chapter Revision Process

Compendial, USP-NF



- If incorporation of comments do not substantially change the content of the proposal, the General Chapter can move forward through the compendial process to be published in the *USP-NF*.
- After approval, the Commentary is posted on the uspnf.com website once the General Chapter is also published
- General Chapter proposal, with or without changes, is submitted to the Publications department to prepare the document for publication in the USP-NF.
- The final proposal is presented to the corresponding EC through official meetings and is then voted on to become official (termed "balloting").
- The proposal is released/posted in the *USP–NF* and becomes official six months later. The publication schedule is provided at https://www.uspnf.com/publication-comment-schedule

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2

Focus on 3 R's

Focus on 3R's: Alternatives to Animal testing

The industry and regulators show their support for the 3Rs, USP has kept pace, with no shortage of notable efforts to advance the 3Rs:

2014
USP ⟨126⟩ *Somatropin Bioidentity Tests* w/animal & non-animal testing option

2018
USP published "the first cell-based assay for insulin products … [which] will provide an alternative to the animal assay."[1]

2019
100+ attendees at USP workshop on "Future of Endotoxins and Pyrogen Testing."[2]

2020
USP published a *PF* Stimuli Article with proposed 3R revisions to chapters ⟨87⟩, ⟨88⟩, and ⟨1031⟩ on biological reactivity

2021
USP proposed deletion of the in vivo test in chapter <126> in *PF47(4)*

Sources: [1] USP (2018); [2] Tirumalai & McCullough (2019);

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A review of USP's toxicity animal testing activities by AltTox.org in 2007 found 8 testing areas of concern

Animals still used by USP in our standards (as of 2007) included: Rabbit, Horseshoe crabs, Mice, and Guinea Pigs, thus showing that we still had plenty of 3R growth opportunities:[1]

Rabbit Pyrogen Test (USP)
- Animal: Rabbit;
- Purpose: detect contaminants

Limulus Amoebocyte Lysate Test (USP)
- Animal: Horseshoe crabs;
- Purpose: detect bacterial endotoxin contamination

General Safety Test (USP)
- Animal: Mice
- Purpose: evaluate systemic toxicity

Biologics Safety Test (USP)
- Animal: 2 mice, 2 guinea pigs
- Purpose: Evaluate biologics for toxicity

Mouse Systemic Injection Test (USP).
- Animal: Mice
- Purpose: assessing adverse systemic effects

Rabbit Intracutaneous Reactivity Test (USP)
- Animal: Rabbits
- Purpose: test irritant effects of toxic leachable substances

Rabbit Intramuscular Implantation Test for 1 to 52 weeks (USP)
- Animal: Rabbits
- Purpose: test acute or long-term toxicity of leachable substances

In Vivo Biological Reactivity Tests for Plastics (USP)
- Animal: Mouse, Rabbit
- Purpose: evaluate the biological response to plastics

Source: [1] AltTox.org (2007);
Image credit: horseshoe crab by Hey Rabbit from the Noun Project

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Biological Reactivity Chapters

Proposed changes include the inclusion of Cytotoxicity Tests and Genotoxicity Tests to expand in-vitro testing options.

How USP has tried to balance public health and animal welfare on our Biological Reactivity Chapters, 1960 – 2020:[1]

1960s

- Concern grew over biological effects of packaging

1965

- *Biological Tests — Plastics Containers* became official in response in USP XVII (later, "Biological Reactivity Tests, In Vivo ⟨88⟩")

1990

- USP added *Biological Reactivity Tests, In Vitro ⟨87⟩* with a companion chapter (⟨1031⟩), with the intent of the latter guiding decisions on whether animal testing are actually needed. In practice, however, the most stringent became used as the default.

2020

- USP published a stimuli article in PF to ask for feedback on 3Rs-friendly revisions to ⟨87⟩, ⟨88⟩, and ⟨1031⟩, such as giving more 3R options and more risk discussion.

Sources: [1] USP (2020);

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From Pyrogen <151> to <85> BET:
From 1985-1990, 185 fewer monographs use rabbit test

1942

- USP Chapter <151>, "Pyrogen Test" is added to USP 12

1940s - 1970s

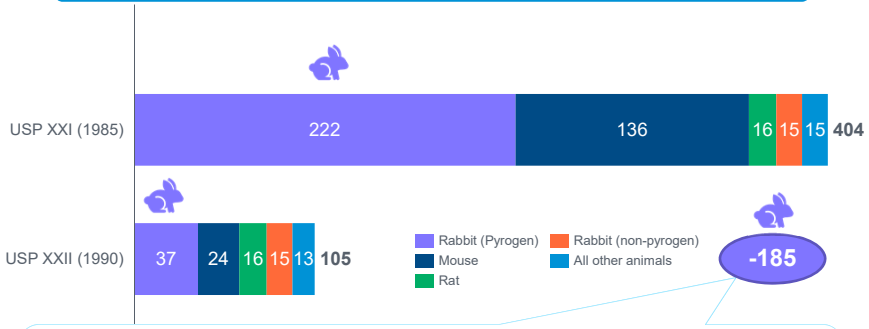
- Pharma industry euthanizes 100K+ rabbits yearly to protect human health.[2]

1980

- USP Chapter <85>, "Bacterial Endotoxins Test" is added to USP 20, drastically reducing the number of rabbits used[1]

1985-1990:

Number of USP documentary standards with animal testing dropped from 404 to 105 between 1985 and 1990, including 185 fewer for rabbits (pyrogen):[1]



"**USP's goal is to replace all rabbit pyrogen tests with the Bacterial Endotoxin Test (BET)** provided that the BET can be validated for specific monographs… **The pyrogen test has been deleted from over 400 monographs, and replaced with the BET test already.**"[1]

Sources: [1] Underhill (1994); [2] Maloney (2018: 2);

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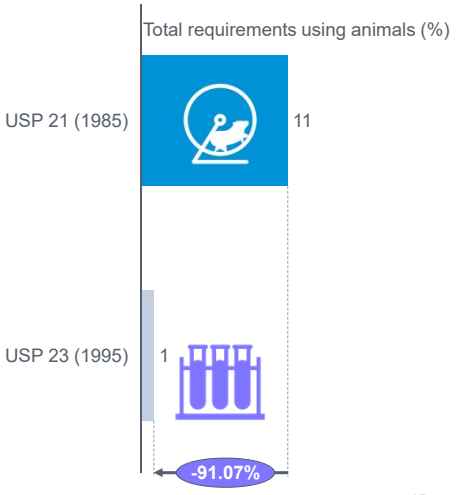
From 1985-1995, USP decreased our animal testing requirements by about 91%

Additionally, USP 23 was able to point to several 3R successes over this 10-year period:

"**Use of Live Animals in Pharmacopeial Testing-**Work began in the 1985-1990 revision cycle on replacement, reduction, and refinement of tests and assays that specified the use of live animals. A 1990 USP Convention Resolution supports this effort as rapidly as science and technology will permit. **Extensive accomplishment of this goal was achieved for USP 23. Whereas 11.2% of total requirements in USP XXI used animals, less than 1% now do in this volume."**[1]

- Other notable successes cited in USP 23 included:
  - **"replacement of 250 rabbit pyrogen tests** by the Bacterial Endotoxins Test, an in vitro procedure."
  - "**mouse safety test was deleted from all antibiotic monographs**."
  - "Testing of ophthalmic products now specifies **cell culture rather than the test for rabbit-eye irritancy**, which was deleted in preparing USP 23."
  - **"Various bioassays also were replaced**."[1]



Sources: [1] USP 23 NF 18 (1995: liv[56])

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Current and recently omitted monographs

Require <151>

Title	Official Status	Official Date
Amitriptyline Hydrochloride Injection	No Longer Official	Omitted 01-Aug-2018
Cefmenoxime for Injection	No Longer Official	Omitted 01-Nov-2020
Cefmenoxime Hydrochloride	No Longer Official	Omitted 01-Nov-2020
Cefotiam for Injection	No Longer Official	Omitted 01-Dec-2020
Cefotiam Hydrochloride	No Longer Official	Omitted 01-Dec-2020
Cefpiramide	No Longer Official	Omitted 01-Dec-2020
Cefpiramide for Injection	No Longer Official	Omitted 01-Dec-2020
Sodium Sulfate Injection	No Longer Official	Omitted 01-May-2022
Ammonium Molybdate Injection	Official	31-Dec-2012
Antithrombin III Human	Official	01-Aug-2022
Floxuridine	Official	01-May-2020
Floxuridine for Injection	Official	01-May-2020
Fluorescein Injection	Official	01-May-2019
Indium In 111 Oxyquinoline Solution	Official	31-Dec-2012
Oxacillin Injection	Official	01-Dec-2021
Polymyxin B for Injection	Official	01-Jan-2018
Polymyxin B Sulfate	Official	01-May-2017
Sulfamethoxazole and Trimethoprim Injection	Official	31-Dec-2012
Trace Elements Injection	Official	31-Dec-2012
Verteporfin for Injection	Official	01-May-2020

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3

History and background

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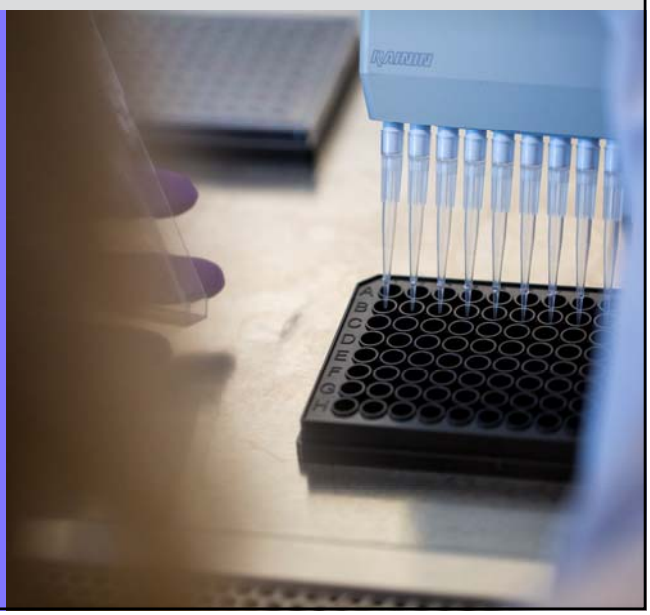
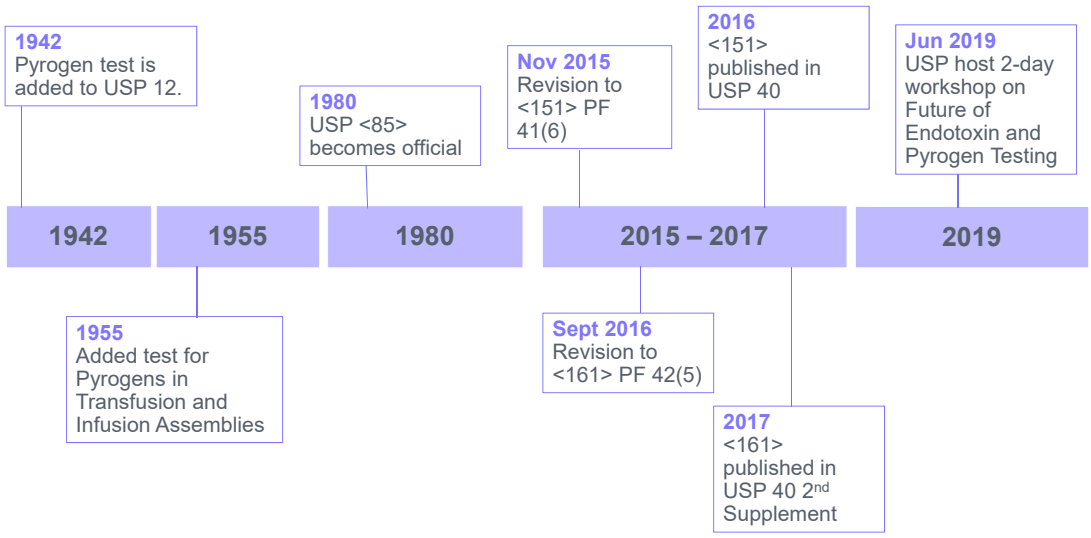
Pyrogenicity Tests

- Pyrogenicity has been associated with infections since the 6th Century BC
- Siebert established the rabbit as the preferred model for pyrogens detection in 1923
- The Rabbit Pyrogens Test was introduced in the 12th revision of USP (1942)
- Nearly 50 years have passed since LAL testing was accepted
- Alternative tests have been suggested
- Questions have been raised about the standards



Pyrogen Tests Timeline



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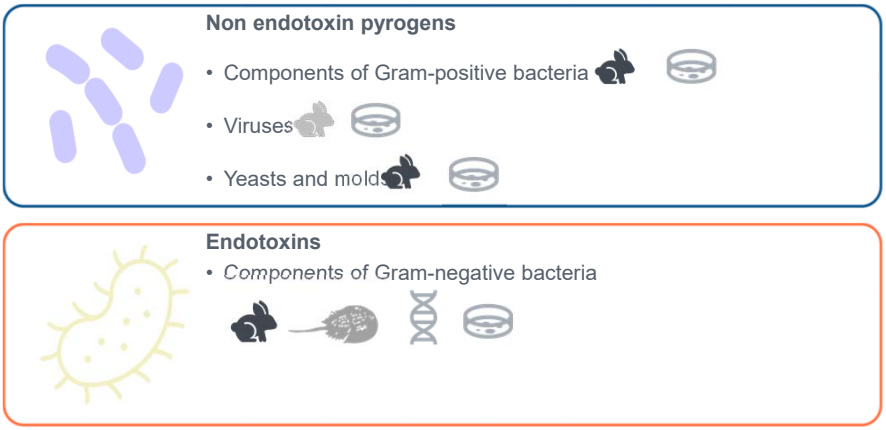
Overview of Pyrogen Tests



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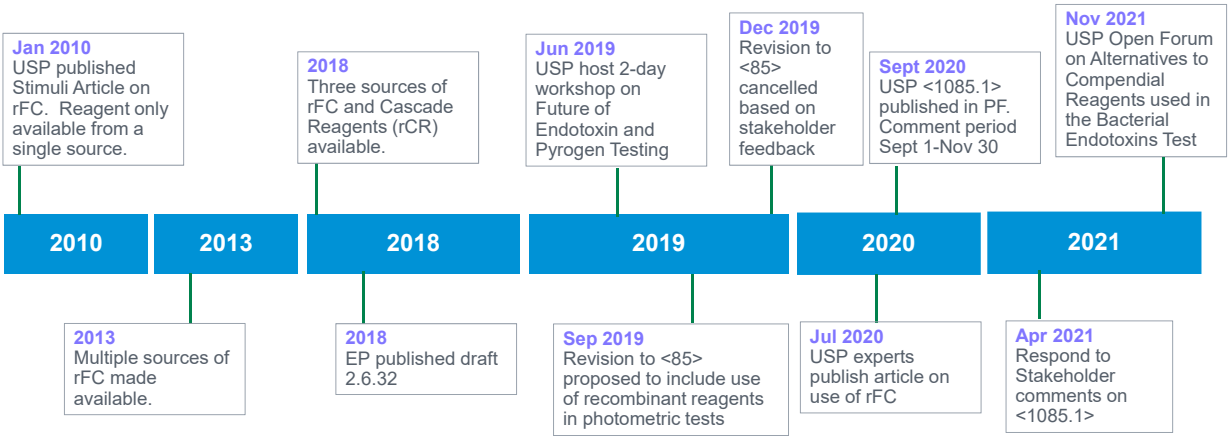
Endotoxin Timeline



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Comparison of Pyrogen Tests

- **Rabbit Pyrogen Test (RPT)**
  - lower sensitivity compared to a human
- **Limulus Amebocyte Lysate (LAL)**
  - not a pyrogen test and reacts very differently to LPS compared to the human immune response
- **Monocyte Activation Test (MAT)**
  - able to detect different kinds of pyrogens

Pyrogen	MAT	RPT	LAL
Endotoxin	+	+	+
Non-endotoxin	+	+	-
Human-specific	+	-	-
Yeasts & molds	+	+	-
Virus	+	+/-	-

References: Lin et al., 2011

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4 Looking to the future

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Alternative tests/Monocyte Activation Test

Regulatory framework

MAT was mentioned by FDA guidance for industry in 2012 as an alternative method for Pyrogen Detection

- **USP <151> (Pyrogen Test)** mentions:
  - "A validated, equivalent in vitro pyrogen or bacterial endotoxin test may be used in place of in vivo rabbit pyrogen test, where appropriate."
  - Effective since May 1, 2017

MAT was introduced in European Pharmacopeia in 2010 as a compendial method, an alternative to RPT: Chapter 2.6.30 – Monocyte Activation Test

- **Chapter 2.6.8 Pyrogens:** Recommendation to replace RPT by MAT wherever possible and after product specific validation.
- **Chapter 5.1.10 Guidelines for using the test for Bacterial Endotoxins**
  - Recommendation is given to perform a risk assessment when using the BET as a pyrogenicity test, due to potential for contamination by non-endotoxin pyrogens: NEP-exclusion by MAT.
  - Reference is made to the use of rFC as alternative to LAL in order to avoid the use of endangered animal species.

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Alternative Methods

Validation of Alternative Methods

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** and must **produce comparable results** to the compendial method or procedure within allowable limits established on a case-by-case basis."

- Demonstrate analytical capability
  - <1225>
  - Specificity, sensitivity, linearity, ruggedness, robustness
- Suitability
  - <85> test for interfering factors
- Comparable results

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Potential Issues for MAT

Endotoxins and Pyrogens 2019 Workshop

Issue	Response
Variable response to stimuli	Use qualified pooled cryopreserved Peripheral Blood Mononuclear Cells (PBMC)
No information about the contaminant	A positive result indicates the presence of a contaminant, but tools are available to help identify the contaminant
Clinical significance of elevated readout is unknown	Studies have shown that rises in IL-6 correlate with rises in body temperature
MAT is supposed to replace both RPT and BET	MAT has the potential to replace RPT but not completely replace the BET

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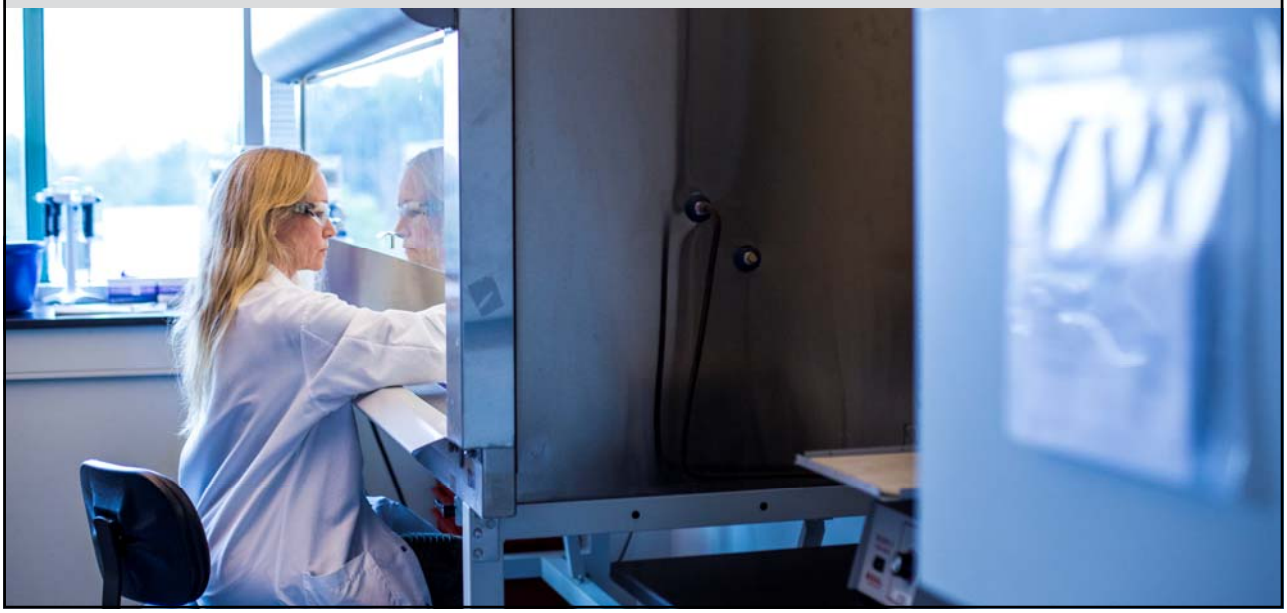
Conclusion



Microbiology Expert Committee

2020 – 2025 Workplan

- Focus Areas
  - Endotoxins and Pyrogens
  - Rapid Microbial Methods
  - Nonsterile Products
  - Microbiological Control of Cell and Gene Therapy Products
  - Sterility Assurance
  - Sterilization and Aseptic Processing

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Opportunities for collaboration

Influence and shape

new and existing standards by sharing your expertise through USP workshops, stakeholder forums, and roundtables

Impact

the evolving landscape of biopharma quality and innovation by collaborating with other leading industry and global regulatory organizations

Get recognized

for your expertise by volunteering on a USP expert committee

Learn

from other pioneering organizations by sharing challenges and best practices

Get early access

to new potential standards by engaging with USP in multi-laboratory collaborative studies

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Thank You

The standard of trust

Stay Connected

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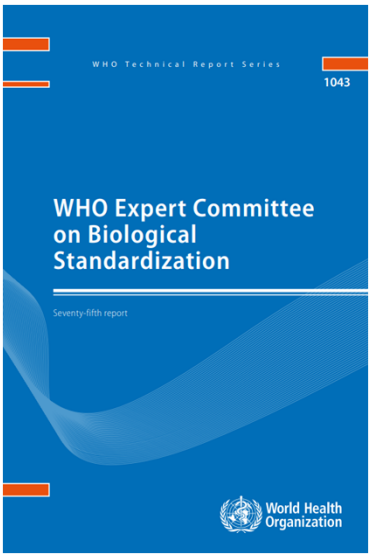
Pyrogenicity testing recommendations in WHO guidelines

Richard Isbrucker, WHO, Norms & Standards for Biologic Products (NSB)

The future of pyrogenicity testing, 14-16 Feb 2023

WHO Guidelines & Recommendations : Some context

WHO guidance documents



WHO Guidance documents related to the quality, safety and efficacy of vaccines and biological therapeutics:

- Provide key principles and specifications which regulators may use to set national requirements
- Meant to complement existing national and international regulations/guidelines and provide guidance where none may exist
- Specifications are also used for WHO prequalification program
- Improve harmonization on product specifications
- Written by international experts invited to join a drafting group members
- All guidance documents are subject to public consultation processes
- Reviewed and adopted by the WHO Expert Committee on Biological Standardization (ECBS) and published as Annexes to the WHO Technical Report Series (TRS)

WHO Guidelines & Recommendations

Vaccine Specific (52)

Annex 3

Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated)

Biotherapeutics & Biosimilars (7)

Annex 4

Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use

Replacement of Annex 3 of WHO Technical Report Series, No. 822

General guidance (22)

Annex 2

Guidelines for independent lot release of vaccines by regulatory authorities

3Rs review of WHO Guidelines & Recommendations

3Rs Project background :

In 2019 a project was proposed to ECBS to review their guidelines for recommendations regarding animal testing

The purpose of this project is to determine:

- Which animal tests are recommended in WHO guidance documents for the quality control and batch release testing of vaccines and biological therapeutics?
- What 3Rs strategies are currently available that are not considered within those guidance documents?
- What are the needs and barriers to better adoption of 3Rs by NRAs/NCLs and manufacturers in the quality control and batch release testing of these products?
- What strategy or response by WHO would be helpful in promoting the adoption of harmonized animal-free methods and/or implementation of 3Rs principles by NRAs/NCLs and manufacturers?

3Rs Project background :

World Health Organization

In Scope	Out of Scope
• Review of publicly available WHO guidance documents for vaccines and biological therapeutics (those adopted by ECBS) • Methods used in their quality control and batch release testing • All 3Rs (i.e. Refinement, Reduction and Replacement) • Identification of barriers towards adopting 3Rs strategies in the quality control and lot release of vaccines and biological therapeutics	• Documents not publicly accessible, which are not considered by ECBS, or are non-WHO guidance documents • Animal methods not related to the QC of vaccines and biological therapeutics (e.g. during product development) • Development or validation of 3Rs methods • Ethical review of the use of animals • Non-constructive criticisms of WHO, member states, NRAs/NCLs, or manufacturers

3Rs Project background (Stage 1):

World Health Organization

Review and Recommendations (Audit):

3-year timeline (2020 - 2023)

Led by an external agency (UK NC3Rs)

- Avoid potential bias inherent in self-reviews
- Manage the project and deliver the final report
- Establish international working group, and focus groups (WHO is a participant)
- Organize workshops / meetings
- Conduct survey of NRA/NCLs and manufacturers

3Rs Project background (Stage 2):

Response and Implementation:

Led by WHO / NSB in consultation with ECBS

Dependent on outcomes and recommendations in final report from Stage 1 provided by NC3Rs

- Recommendations should be based on sound scientific principles
- Supported by findings from the surveys
- Suggested revisions to the texts/3Rs language to be provided for each guidance document where relevant
    - Adoption of the suggested texts to be subject to WHO drafting processes as per all revisions to guidelines

Progress to date :

81 guidance documents reviewed (dating from 1972 – 2022)

350 animal tests for QC / lot release testing identified in 61 guidelines

5 thematic test categories emerged from the review and focus groups were formed to draft proposed revisions to the text:

- Potency/immunogenicity testing
- Endotoxin and pyrogenicity testing
- Neurovirulence testing
- Adventitious agent testing
- Specific toxicity testing

Alternative text emphasising 3Rs for most tests finalised

Endotoxin and Pyrogenicity testing recommendations in WHO guidance documents

Findings from guideline review :

Product	Year	Endo/ LAL	Pyro	MAT
Meningococcal PS unconjugated	1975		X	
Rift Valley fever vaccine	1981		X	
Human interferons	1989		X	
Typhoid PS, unconjugated	1994		X	
Haemorrhagic fever vaccine	1994		X	
Hepatitis A vaccine	1995	X		
Tick-Bourne encephalitis vaccine	1999		X	
Haem. influenza b (Hib) vaccine	2000	X	X	
Men C conjugate vaccine	2003	X	X	
Smallpox vaccine	2004	X		
Whole-cell pertussis vaccine	2007	X	X	
Rabies vaccine	2007		X	
Japanese encephalitis, inactive	2011		X	
Men A conjugate vaccine	2011	X	X	
Pneumococcal conjugate vaccine	2013	X	X	
Influenza, live vaccine	2013	X		
Hepatitis B vaccine	2013	X	X	

Product	Year	Endo/ LAL	Pyro	MAT
Yellow fever vaccine	2013	X		
Acellular pertussis vaccine	2013	X		
Japanese encephalitis, live vaccine	2014	X		
DT-based combo vaccines	2014	X	X	
Malaria vaccine	2014	X	X	
Human Papillomavirus vaccine	2016	X	X	X
Snake antivenom IgG	2017	X	X	
Influenza, inactivated, vaccine	2017	X		
Ebola vaccine	2018	X	X	X
Hepatitis E vaccine	2019	X	X	X
RSV vaccine	2020	X	X	X
Polio, inactivated, vaccine	2020	X		
Typhoid conjugate vaccine	2021	X	X	X
Enterovirus 71 vaccine	2021	X		
mRNA vaccines	2022	X	X	X
mAbs production	2022	X	X	X

Evolution of language around pyrogenicity testing :

1975:
Requirements for Meningococcal Polysaccharide Vaccine (TRS 594)

5.5.1 *Pyrogenicity test*

Each filling lot shall be tested for pyrogenicity by the intravenous injection of rabbits. Three or more healthy rabbits that have not been injected previously shall be used. The vaccine, reconstituted in the form in which it is to be used, shall be diluted further in pyrogen-free physiological saline so that each rabbit shall receive, by injection into the ear vein, the following doses of dry weight polysaccharide per kilogram of rabbit weight :

Group A vaccine, 0.0025 µg
Group C vaccine, 0.0025 µg
combined Groups A and C vaccine, 0.0050 µg

In each instance the specified dosage level of polysaccharide for each rabbit shall be suspended in 1 ml of physiological saline per kilogram of rabbit weight. The criteria for passing the test shall be those specified in the International Pharmacopoeia.[3]

Evolution of language around pyrogenicity testing :

1989:
Requirements for human interferons prepared from lymphoblastoid cells (TRS 786)

10.2 Tests for pyrogenic substances

The pyrogen content of the final bulk shall be determined by a method agreed with the national control authority.

1999:
Requirements for tick-borne encephalitis vaccine (TRS 889)

A.6.5 ***Pyrogenic substances***

Each final lot shall be tested for pyrogenic substances. The test shall be approved by the national control authority.

Evolution of language around pyrogenicity testing :

2016:
Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines (TRS 999)
2019:
Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines (TRS 1016)

A.9.7 **Test for pyrogenic substances**

Each final lot should be tested for pyrogenic substances. Where appropriate, tests for endotoxin (for example, the limulus amebocyte lysate (LAL) test) should be performed. However, where there is interference in the test – for example, because of the addition of an immunostimulant such as MPL – a test for pyrogens in rabbits should be performed.

A suitably validated monocyte-activation test may also be considered as an alternative to the rabbit pyrogen test.

The test is conducted until consistency of production is demonstrated, subject to the agreement of the NRA.

Evolution of language around pyrogenicity testing :

2022:

Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use (TRS 1043):

A.8.2.13 Endotoxin or pyrogen content

The endotoxin content of each lot of the final product should be consistent with levels found to be acceptable in product lots used during clinical trials. Suitable in vitro methods include the test for bacterial endotoxins using recombinant factor C or the LAL test. The test selected for assessing endotoxin content must be validated for its intended purpose.

The need for pyrogenicity testing should be determined during the manufacturing development process based on an appropriate risk assessment. This may need to be re-evaluated following any changes in the production process or relevant reported production inconsistencies that could influence the quality of the product with regard to its pyrogenicity. A monocyte activation test may be used for monitoring the potential pyrogenic activity in the final product after a product-specific validation. Although a rabbit pyrogenicity test may be accepted by the NRA, its use is discouraged due to the inherent variability, high re-testing rates and interspecies differences in pyrogenic responses compared to humans.

Evolution of language around pyrogenicity testing :

Following review of the guidelines, the pyrogenicity focus group has drafted text to recommend for replacing the endotoxin and pyrogenicity sections of existing and future guidelines:

- Risk-based approach should be used during product development, and following relevant manufacturing changes or OOS/inconsistencies, to determine the need for endotoxin/pyrogenicity testing
- If only endotoxin, then use recombinant Factor C or LAL (preferably rFC)
- If non-endotoxin pyrogens, then use a MAT in a format appropriate for the product
- Only use the rabbit pyrogenicity test if no other option is possible.

The report from NC3Rs will be presented to ECBS in October 2023. The proposed recommendations to the text for pyrogenicity testing will be reviewed by WHO drafting group(s)

Acknowledgements :

UK NC3Rs:
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Pyrogenicity focus group:
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Etna Marilena Paola
Octavio Presgrave
Shahjahan Shaid
Paul Stickings
Caroline Vipond

Monocyte Activation Test (MAT) in Chinese Pharmacopoeia

EDQM-EPAA Hybrid Event on Pyrogenicity

Dr. Qing He

National Institutes for Food and Drug Control, China

14-16 February 2023, Brussels, Belgium

中国食品药品检定研究院

National Institutes for Food and Drug Control

Outline

1. A Brief Self-introduction
2. Development of Pyrogen and Endotoxin tests in ChP
3. Latest Development of *In Vitro* Pyrogen Tests in ChP

Slide 2

National Institutes for Food and Drug Control (NIFDC)

- A subsidiary of National Medical Products Administration (NMPA)
- Center for Medical Device Standardization Administration NMPA
- China National Institutes for Drug Conrol

Slide 3

International Role and Cooperation

- WHO Collaborating Centre for Standardization and Evaluation of Biological Products
- Establish long-term cooperation mechanism with international authoritative counterparts
- Participate in the establishment and collaborative research of WHO IS

Dr. Junzhi Wang, Director of WHO CC

Updated MOU with NIBSC, 2008

Updated MOU with PEI, 2010

Subscribed MOU with CVE, 2010

Slide 4

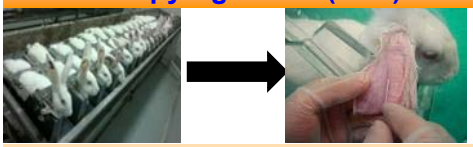
Pyrogen—A Key Parameter Affecting The Safety of Products

- Pyrogen including endotoxin and non-endotoxin pyrogen
- Pyrogen testing is a statutory requirement of pharmacopoeias to control the safety of parenteral drugs
- The research of feasible pyrogen tests is highly concerned by pharmacopoeias

Slide 5

Development of Pyrogen and Endotoxin Tests in Chinese Pharmacopoeia

- Rabbit pyrogen test (RPT): firstly adopted by 1953 edition of ChP



- has variations in responses
- involves the use of animals *in vivo*
- expensive

- Bacterial endotoxin test (BET): firstly adopted by 1990 edition of ChP

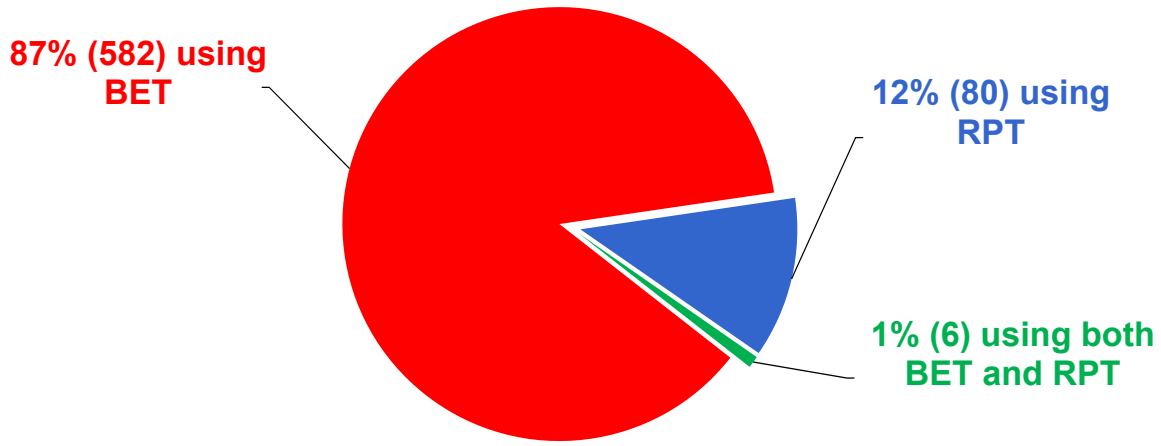
- can only detect gram-negative bacteria endotoxins
- horseshoe crabs are the second-class protected animal in domestic

- Monocyte activation test (MAT): firstly adopted by 2020 edition of ChP

- based on the mechanism of fever reaction
- involves the use of cells *in vitro*
- can detect endotoxin and non-endotoxin pyrogens

Slide 6

Products using Pyrogen and Endotoxin Tests in ChP



Slide 7

Overview of MAT in Chinese Pharmacopoeia

Role of MAT	☐ only used as a supplementary method for pyrogen test
Design of MAT	☐ quantitative test (corresponding to the method A of EP)
Version of MAT	☐ PBMC—IL-6
	☐ Fresh whole human blood—IL-1β/IL-6
	☐ Cryopreserved human blood—IL-1β/IL-6
	☐ Mononuclear cell line HL60—IL-6

The general principle 9301 "Guidelines for the application of safety tests for Injections", Vol Ⅳ of 2020 Chp.

Slide 8

Domestic Validation on MATs

➢ Trained by experts of PEI

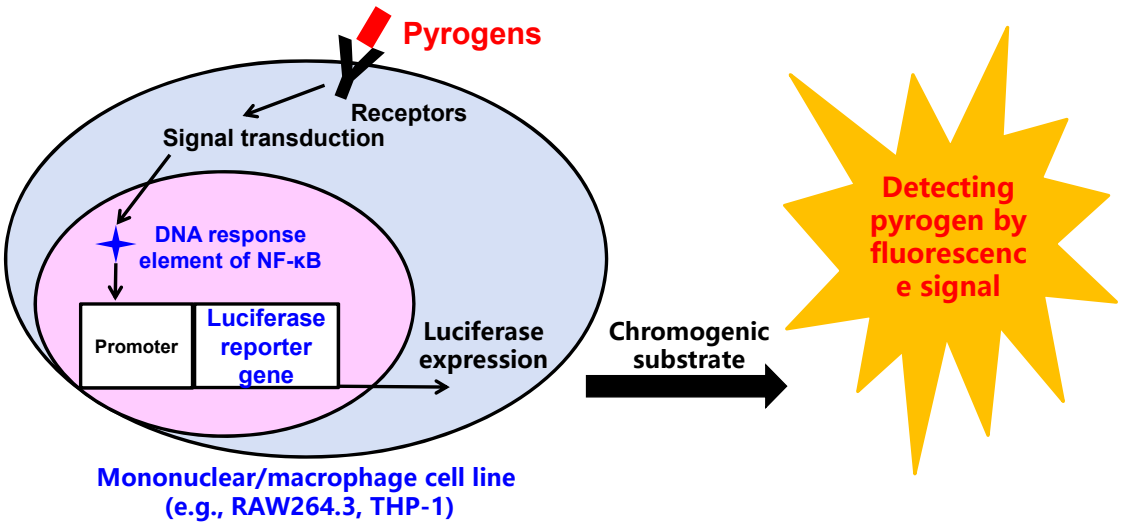
➢ Trained by experts of NIBSC

MATs	Within-lab reproducibility (%)	Inter-lab reproducibility (%)	Sensitivity (%)	Specificity (%)
PBMC—IL-6	86.7~100	78.5~96	90.1	92.3
Cryo pooled human whole blood—IL-1β	80.0~86.7	63.6~85.7	82.5	100
Cryo pooled human whole blood—IL-6	86.7~100	57.1~92.9	81.7	100

Chin J Pharm Anal, 2012, 32(10): 5-11. Innate Immun, 2018, 24(5):316-322.

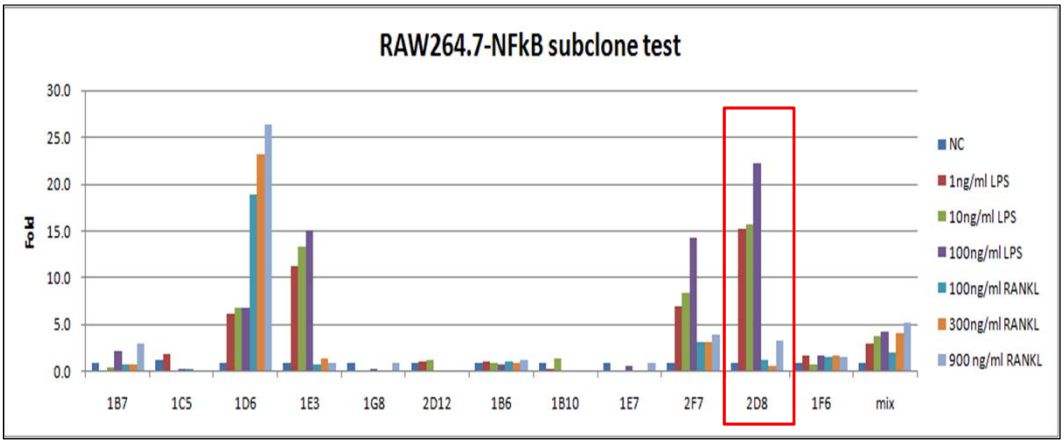
Slide 9

Another Version of MAT—A Reporter Gene Assay for Pyrogen Detection



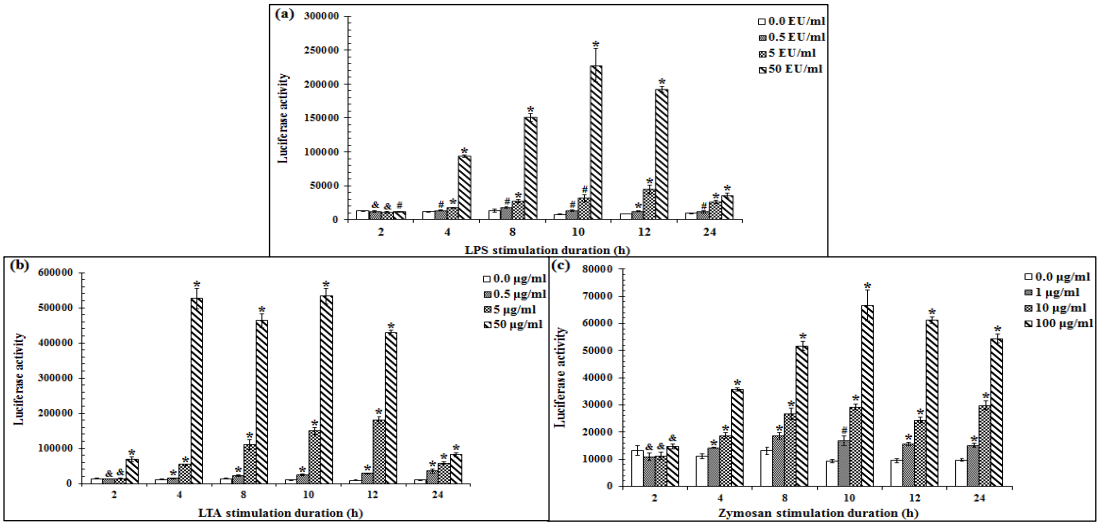
Slide 10

Construction and Screening of RAW246.7-NF-κB Subclone: √ 2D8



Slide 11

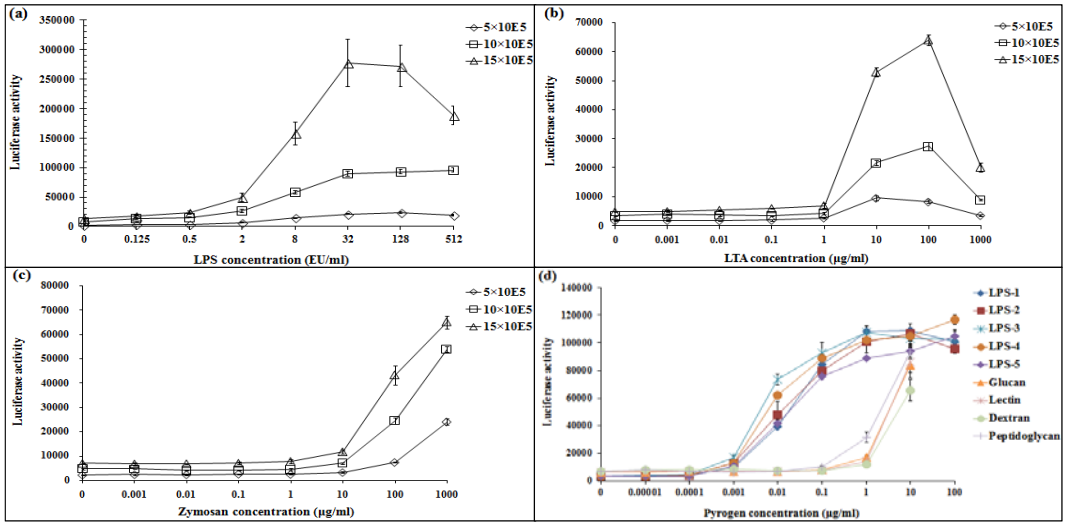
The Time-Effect Relationships of Pyrogens activating NF-κB: √ 10h



Jpn J Infect Dis. 2020, 73, 111–118.

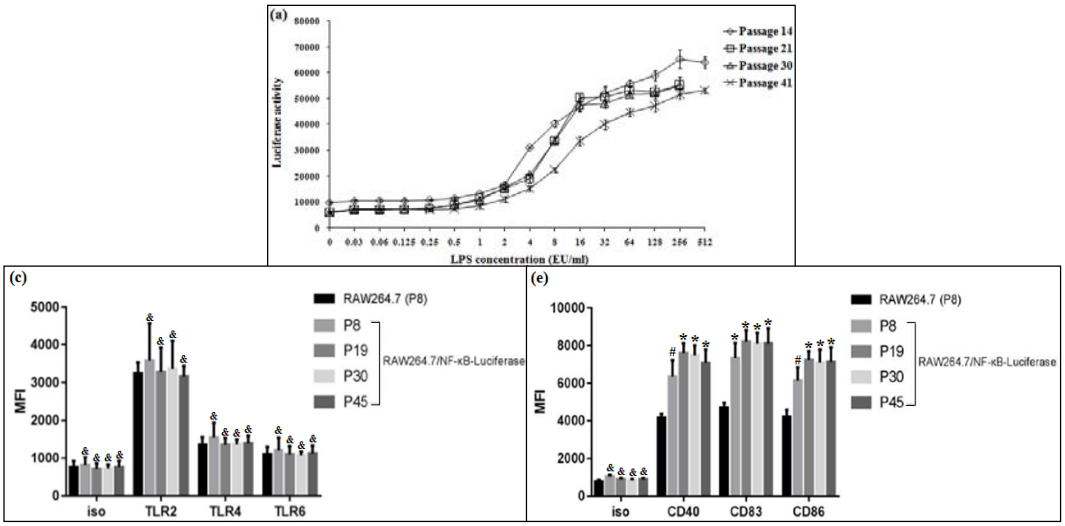
Slide 12

The Dose-Effect Relationships of Pyrogens activating NF-κB in RAW264.7 Cells at Different Cell Densities: √ 10×10^5



Jpn J Infect Dis. 2020, 73, 111–118. Slide 13

The Dose-Effect Relationships of LPS in activating NF-κB in RAW264.7 Cells and Cell Phenotypes at Different Cell Passages: √ 41



Jpn J Infect Dis. 2020, 73, 111–118. Slide 14

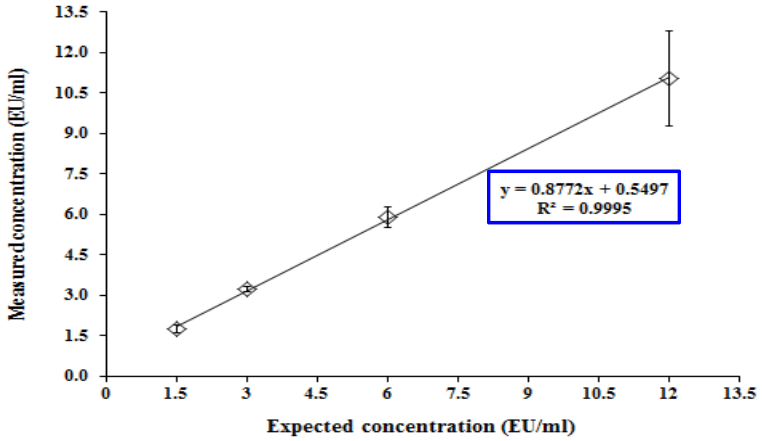
Precision of The Assay for Detecting LPS in The Laboratory

- The intraassay and interassay coefficients of variation (CVs) were generally less than 13% and 16%, respectively.

	Round 1	Round 2	Round 3	Interassay CV (%)
	1.927	1.791	1.717	
Sample 1(EU/ml)	1.879	1.653	1.765	7
	1.725	1.871	1.522	
Intraassay CV (%)	6	6	8	/
	3.319	3.216	3.103	
Sample 2(EU/ml)	3.418	3.265	3.255	3
	3.089	3.258	3.249	
Intraassay CV (%)	5	1	3	/
	5.426	5.678	5.924	
Sample 3(EU/ml)	6.065	5.683	6.755	7
	5.719	5.726	6.175	
Intraassay CV (%)	6	0	7	/
	11.91	8.752	9.632	
Sample 4(EU/ml)	11.646	9.039	12.121	16
	14.372	11.125	10.655	
Intraassay CV (%)	12	13	12	/

Jpn J Infect Dis. 2020, 73, 111–118. Slide 15

Accuracy of The Assay for Detecting LPS in The Laboratory



Jpn J Infect Dis. 2020, 73, 111–118. Slide 16

Application of The Assay to Drugs

➢ **The assay has potential for various applications.**

Drug	Fold-dilution	NF-κB response	
		Spikerecovery (%)	Interference
Nivolumab injection	16	121	no
Rituximab injection	8	125	no
Bevacizumab injection	16	161	no
Etanercept solution for injection	168	105	no
*Haemophilus influenzae*type b conjugate vaccine	400	74	no
23-Valent pneumococcal polysaccharide vaccine	400	75	no
Group A and group C meningococcal conjugate vaccine	8000	85	no
Basiliximab for injection	64	70	no
Diphtheria, tetanus, pertussis (acellular, component), poliomyelitis (inactivated) vaccine	1600	96	no
Imject alumadjuvant	1000	129	no

Jpn J Infect Dis. 2020, 73, 111–118.

Slide 17

Validation of THP-1/NF-κB Test

➢ **The THP-1/NF-κB test has good stability and accuracy in different laboratories.**

Test	Within-laboratory reproducibility (%)	Inter-laboratory reproducibility (%)	Sensitivity(%)	Specificity (%)
THP-1/NF-κB	Lab. 1: 85	Lab. 1—Lab. 2: 83.3	89.9	90.9
	Lab. 2: 80	Lab. 1—Lab. 3: 95.6		
	Lab. 3: 80	Lab. 2—Lab. 3: 86.7		
Rabbit pyrogen test	/	/	57.9	88.3

Data to be published

Slide 18

The Latest Development of *In Vitro* Pyrogen Tests in ChP

- **2020 edition of Chinese Pharmacopoeia supplementary will adopt the reporter gene assay for pyrogen detection.**

https://www.chp.org.cn/gjyjw/swzp/17463.jhtml

Slide 19

Key Points

- **Pyrogen and endotoxin tests are key methods to ensure the safety of products by Chinese Pharmacopoeia.**
- **Regulatory authorities in China have always attached importance to the overall trend of the development of pyrogen/endotoxin test and the animal welfare. In view of the above trend, Chinese Pharmacopoeia is actively establishing novel *in vitro* pyrogen tests such as MAT, the reporter gene assay for pyrogen detection.**
- **In the future, Chinese Pharmacopoeia will focus on promoting the practical application of those *in vitro* pyrogen tests.**

Slide 20

Acknowledgements

- We thank Dr. Junzhi Wang, Dr. Ingo Spreitzer, Dr. Dejiang Tan, Dr. Hua Gao, Dr. Lan Wang and Dr. Chuanfei Yu for their invaluable assistance!

- Thank you for your attention
- Email: heqing@nifdc.org.cn

Slide 21

EDQM-EPAA Pyrogenicity Event

The future of pyrogenicity testing: phasing out the rabbit pyrogen test

14-15 February 2023, Brussels, Belgium

Exploration of the MAT in Japan

National Institute of Health Sciences, Japan
Division of Safety Assessment
Takao Ashikaga

What is NIHS?

The National Institute of Health Sciences (NIHS) conducts testing, research, and studies toward the proper evaluation of the quality, safety, and efficacy of pharmaceutical products, foods, and the numerous chemicals in the living environment.

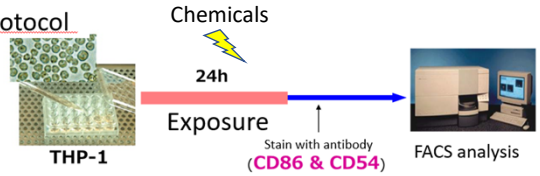
Issues pertaining to human health change with the times. Many new pharmaceuticals, foodstuffs, and substances used in daily living are being created. Given this, the NIHS serves to control the products that are generated by science and technology to make sure that they truly benefit the general public.

https://www.nihs.go.jp/english/

Who is Takao?

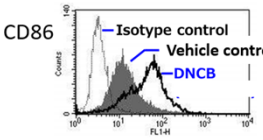
- ✓ Developer of the human Cell Line Activation Test (in vitro skin sensitization test, h-CLAT(OECD442E)), which is a kind of Monocyte Activation Test.
- ✓ Work for JaCVAM (Japanese Center for the Validation of Alternative Methods) and a member of Japanese Pharmacopoeia Biological Testing Committee.

- Protocol



*Ashikaga et al., 2006, *Toxicol. in Vitro* 20(5), 767-773.
Sakaguchi et al., 2006, *Toxicol. in Vitro* 20(5), 774-784.

- Prediction model



Judgement:

CD86 RFI ≥ 150% or CD54 RFI ≥ 200%

RFI: Relative Florescence Intensity

What is Japanese Pharmacopoeia?

Japanese Pharmacopoeia (JP) is established and published to regulate the properties and quality of drugs by the Minister of Health, Labour and Welfare after hearing the opinion of the Pharmaceutical Affairs and Food Sanitation Council (PAFSC).

The JP consists of General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests, Processes and Apparatus and Official Monographs.

Items selected for entry in the JP must be those important in health care based on the necessity of the drug in medical practice, wide application and experience of use.

Since it was first published in June 1886, the JP has been revised many times periodically. The 18th edition of the JP came into effect on June 7, 2021.

https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0009.html

The Japanese Pharmacopoeia 18th edition can be downloaded at the HP of Ministry of Health, Labour and Welfare, Japan.

https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000066597.html

JP is examining the MAT

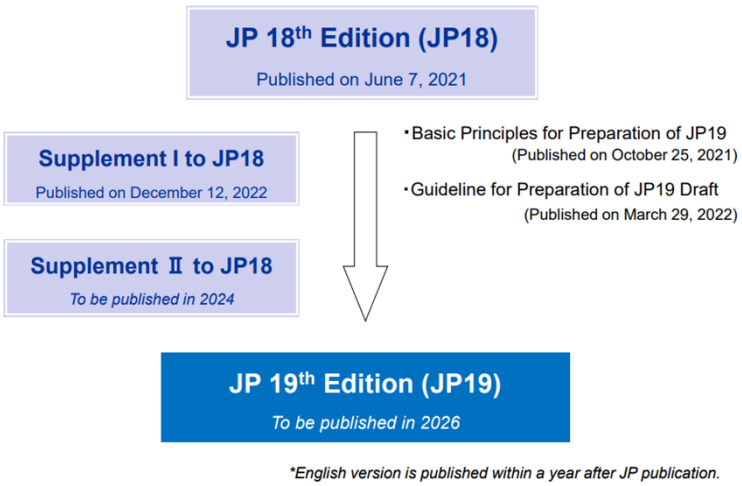
International Harmonization of Pharmaceopoeias is discussed (e.g. Pharmaceopoeial Discussion Group (PDG))

Japanese Pharmacopoeia Biological Testing Committee is

- ✓ examining if a method to detect the presence of pyrogens using human blood or cells as an alternative to the Japanese Pharmacopoeia 18 general tests ``4.04 Pyrogen Test'' should be introduced.
- ✓ referring to the Ph. Eur. Chapter 2.6.30 (Monocyte Activation Test).
- ✓ investigating the status of domestic and overseas utilization of the MAT, and investigating the necessity of inclusion.

Next edition will be published in 2026

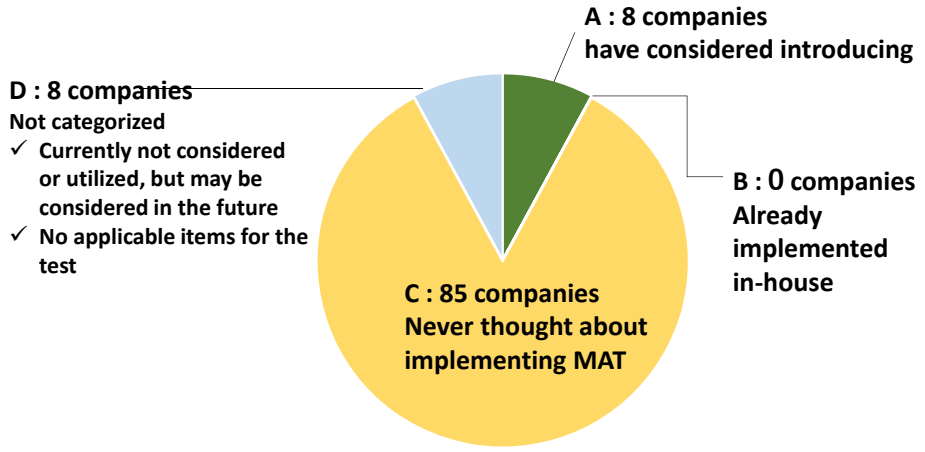
Publication Schedule of Japanese Pharmacopoeia



*English version is published within a year after JP publication.

https://www.pmda.go.jp/files/000242348.pdf

Current status of utilization of MAT in Japanese pharmaceutical companies



✓ JP Biological Testing Committee conducted a survey and 101 compa
✓ Over 80% of Japanese companies have never considered implementin

Points to be confirmed when listing the MAT test in the Japanese Pharmacopoeia

1. The relationship between the pyrogen test and the endotoxin test (LAL test)
2. Differences in reactivity between peripheral blood-derived monocytes and monocytic cell lines and how to select methods (which method is better?)
3. When using peripheral blood-derived monocytes, are single-donor monocytes or pooled monocytes preferable? Is there a recommendation for the number of pooled donors for pooled monocytes? (In Japan, it is difficult to obtain human peripheral blood for commercial purpose).
4. When using peripheral blood-derived monocytes or monocytic cell lines, detailed validation items that must be performed when setting test methods should be provided.

Points to be confirmed when listing the MAT test in the Japanese Pharmacopoeia

5. Whether only endotoxin reference is sufficient?

6. Which non-endotoxin reference should be used?

7. Non-endotoxin standard products should be included in commercial kits.

8. Stability of supplying of reagents (multiple kits should be available)

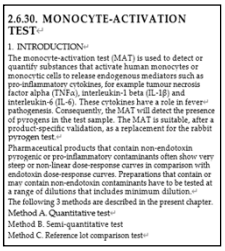
Discussion in the Committee

Discussion on the MAT

What is the MAT?
How to do the MAT?
Understand the current Ph. Eur. Chapter 2.6.30 (Monocyte Activation Test)

Discussion on a draft JP MAT

- ✓ Structure
- ✓ Volume
- ✓ Scientific validity
- ✓ International harmonization



2.6.30. MONOCYTE-ACTIVATION TEST

1. INTRODUCTION

The monocyte-activation test (MAT) is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators such as pro-inflammatory cytokines, for example tumour necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6). These cytokines have a role in fever pathogenesis. Consequently, the MAT will detect the presence of pyrogens in the test sample. The MAT is suitable, after a product-specific validation, as a replacement for the rabbit pyrogen test.

Pharmaceutical products that contain non-endotoxin pyrogenic or pro-inflammatory contaminants often show very steep or non-linear dose-response curves in comparison with endotoxin dose-response curves. Preparations that contain or may contain non-endotoxin contaminants have to be tested at a range of dilutions that includes minimum dilution.

The following 3 methods are described in the present chapter.

Method A. Quantitative test

Method B. Semi-quantitative test

Method C. Reference lot comparison test

Discussion has been conducted from many aspects like describe above.

Long way to publish …

Ph. Eur. Chapter 2.6.30 is being revised

PharmEuropa - Revision of Chapter 2.6.30 Monocyte Activation Test

Current development

However, even today the development of alternative methods continues and the corresponding monographs are also revised again and again. Accordingly, the EDQM has currently published a revision of Chapter 2.6.30 Monocyte Activation Test in Pharmeuropa 34.2 with a public deadline of 30 June 2022.

As an example, the previously differentiated methods A, B and C disappear. Methods A and B have been combined into a single semi-quantitative test called "Method 1". Method C is now referred to as "Method 2".

NEWS
Register now for ECA's GMP Newsletter
Newsletter registration

In addition, regarding concentrations and product dilutions, a new section has been added that provides recommendations on how to present endotoxin-equivalent concentrations in the MAT and explains how the use of per-sample or per-assay concentrations affect the reported sensitivity value of the assay.

The EDQM also notes that: *"This revision is also part of a wider effort to remove the rabbit pyrogen test from the Ph. Eur. which will be announced in a forthcoming issue of Pharmeuropa:"* (also see the EDQM newsroom report entitled European Pharmacopoeia to put an end to the rabbit pyrogen test). In this context, references to the rabbit pyrogen test have been removed from the introduction and from the guidelines.

ECA Academy News (18.05.2022): https://www.gmp-compliance.org/gmp-news/pharmeuropa-revision-of-chapter-2-6-30-monocyte-activation-test

Exchanges opinions and information between Japan and Europe are necessary from the perspective of international harmonization!

JaCVAM

1. JaCVAM is evaluating the PyroMAT according to a manufacturer's request.

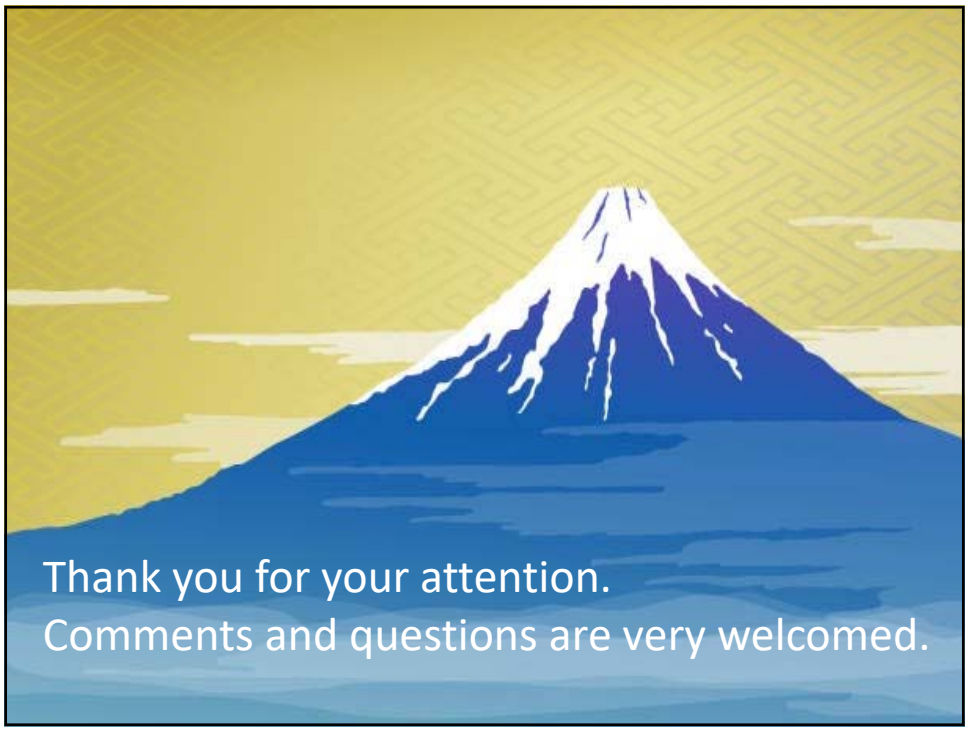
https://www.merckmillipore.com/JP/ja/product/PyroMAT-kit,MM_NF-PYR0MATKIT

2. JaCVAM is conducting a validation study on a Japanese MAT kit named MylcMAT.

- ✓ Animal free system (synthetic medium used)
- ✓ Confirmed high sensitivity to o variety of non-endotoxin pyrogens
- ✓ Stable supply

Conclusion

- ✓ In Japan, Japanese Pharmacopoeia Biological Testing Committee is examining if MAT should be listed in the next JP.
- ✓ Low number of Japanese companies have considered implementing MAT due to several issue (e.g., relationship with the endotoxin test, kit validation, non-endotoxin reference, human peripheral blood, etc.).
- ✓ International cooperation is essential for regulatory acceptance as the technology in this field is rapidly advancing.



PROGRESS IN THE REGULATORY ACCEPTANCE OF MAT IN BRAZIL

Octavio Presgrave
BraCVAM/FIOCRUZ

MAT EXPERIENCE

- 1988 – use of MonoMac-6 (Poole, 1988)
- 2002-2008 – Konstanz University – technology transfer
- 2003 – Octavio Presgrave, M.Sc. – use of cytokine release test
- 2011 – Izabela Gimenes, M.Sc. – MAT for non-endotoxin pyrogens
- 2015 – Cristiane Caldeira, Ph.D. – MAT for hyperimmune sera and air quality
- In course – Ana Beatriz, M.Sc – MAT for COVID-19 vaccines

ARTICLES

- DA SILVA, CRISTIANE CALDEIRA ; DE OLIVEIRA, CAROLINA BARBARA NOGUEIRA ; CARNEIRO, PATRÍCIA DOS SANTOS ; MARENGO, ELIANA BLINI ; DE MATTOS, KATHERINE ANTUNES ; DE ALMEIDA, RICARDO SERGIO COUTO ; SPOLADORE, JANAINA ; ALVES, GUTEMBERG GOMES ; PRESGRAVE, OCTAVIO AUGUSTO FRANÇA ; DELGADO, ISABELLA FERNANDES . Métodos alternativos para a detecção de pirogênios em produtos e ambientes sujeitos a Vigilância Sanitária: avanços e perspectivas no Brasil a partir do reconhecimento internacional do Teste de Ativação de Monócitos. Vigilância Sanitária em Debate: Sociedade, Ciência & Tecnologia, v. 6, p. 137-149, 2018.
- DE MATTOS, KATHERINE ANTUNES ; NAVEGA, E. C. A. ; SILVA, V. F. ; ALMEIDA, A. S. ; Caldeira, C. ; PRESGRAVE, O. A. F. ; GUEDES JUNIOR, D. S. ; Delgado, I. F. . Applicability of the Monocyte Activation Test (MAT) in the Quality Control of the 17DD Yellow Fever Vaccine. ATLA-ALTERNATIVES TO LABORATORY ANIMALS, v. 46, p. 23-37, 2018.
- SILVA, V. F. ; GUEDES JUNIOR, D. S. ; SILVEIRA, I. A. ; ALMEIDA, A. S. ; CONTE, F. P. ; Delgado, I. F. ; Caldeira, C. ; Presgrave OAF ; DE MATTOS, KATHERINE ANTUNES . A comparison of pyrogen detection in the quality control of meningococcal conjugate vaccines: the applicability of the Monocyte Activation Test. ATLA-ALTERNATIVES TO LABORATORY ANIMALS, v. 46, p. 255-272, 2018.
- DA SILVA, CRISTIANE CALDEIRA ; PRESGRAVE, OCTAVIO AUGUSTO FRANÇA ; HARTUNG, THOMAS ; DE MORAES, AUREA MARIA LAGE ; DELGADO, ISABELLA FERNANDES . Applicability of the Monocyte Activation Test (MAT) for hyperimmune sera in the routine of the quality control laboratory: Comparison with the Rabbit Pyrogen Test (RPT). Toxicology in Vitro, v. 32, p. 70-75, 2016.
- GIMENES, IZABELA ; CALDEIRA, CRISTIANE ; PRESGRAVE, OCTAVIO AUGUSTO FRANÇA ; MOURA, WLAMIR CORREA DE ; VILLAS BOAS, MARIA HELENA SIMOES . Assessment of pyrogenic response of lipoteichoic acid by the monocyte activation test and the rabbit pyrogen test. Regulatory Toxicology and Pharmacology, v. 73, p. 356-360, 2015.
- DA SILVA, CRISTIANE CALDEIRA ; CRUZ, MAYARA ; FREITAS, JOÃO CARLOS ; PRESGRAVE, OCTAVIO ; MORAES, AUREA ; DELGADO, ISABELLA FERNANDES . Aplicabilidade do Teste de Ativação de Monócitos (MAT) no Brasil: importância da sua utilização como teste para detecção de pirogênios no controle da qualidade de produtos injetáveis. Vigilância Sanitária em Debate: Sociedade, Ciência & Tecnologia, v. 3, p. 41-46, 2015.

LEGAL EVENTS IN BRAZIL

- CONCEA – National Council for the Control of Animal Experimentation – officialization of NAMs
- 2019 - Normative Resolution n. 45 – recognize MAT as official (limit 2024)
- 2021 - BraCVAM suggests the Brazilian Pharmacopoeia to include MAT as oficial monograph
- 2022 – WG of Brazilian Pharmacopoeia – Cristiane Caldeira (member) – in course

BraCVAM Members (in alphabetical order)

Carolina Bárbara de Oliveira

Claudia da Conceição

Cristiane Caldeira

Elias de Jesus

Jonas Roza

Octavio Presgrave

Wlamir Moura

Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz
Vice-Presidência de Pesquisa e Coleções Biológicas

ICTB
Instituto de Ciência e Tecnologia em Biomodelos

BraCVAM
Centro Brasileiro de Validação de Métodos Alternativos
Brazilian Center for Validation of Alternative Methods

THANK YOU!!!

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EVENT

Joint EDQM-EPAA Hybrid Event on Pyrogenicity

"The future of Pyrogenicity testing: phasing out the rabbit pyrogen test"

14-16th February 2023

Venue: Albert Borschette Conference Centre (CCAB)

Rue Froissart 36, Brussels, Belgium

Disclaimer

The content of this presentation is for informational purpose only. This shall not be treated as an official interpretation of Indian Pharmacopoeia (IP) standard or relied on to demonstrate compliance with IP requirements.

INDIAN PHARMACOPOEIA COMMISSION

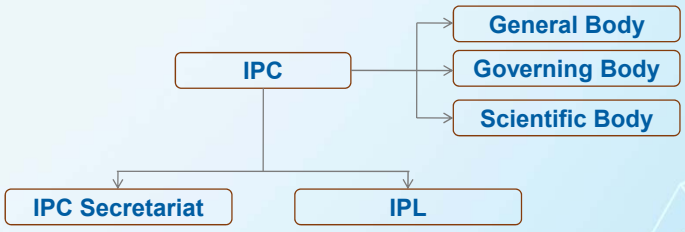
(Ministry of Health & Family Welfare, Government of India)
Sector 23, Raj Nagar, Ghaziabad 201002 (U.P.), India
E-mail: lab.ipc@gov.in; Website: www.ipc.gov.in

15 February, 2022

Pyrogenicity testing- Indian Pharmacopoeia (IP) perspective

Indian Pharmacopoeia Commission (IPC)

- The Govt. of India has created a separate, dedicated, autonomous institution-Indian Pharmacopoeia Commission (IPC) in 2009-to deal with matters relating to timely publication of the Indian Pharmacopoeia (IP) which is the official book of standards for drug included therein, in terms of the Second Schedule to the Drugs and Cosmetics Act, 1940.
- IP specifies the Standards of Quality (identify, purity and strength) of the drugs imported, manufactured for sale, stocked or exhibited for sale or distributed in India.
- IPC has a three-tier policy formulation and execution setup comprising of the General Body, Governing Body and Scientific Body with experts drawn from various Science & Technology areas.



Mandates of IPC

- To publish new edition and addenda of the IP at regular intervals.
- To publish the National Formulary of India (NFI).
- Certification and distribution of IP Reference Substances (IPRS) and Impurity Standards.
- National Coordination Centre (NCC) for Pharmacovigilance Programme of India (PvPI)
- To establish working relations with National and International Institutes.
- To organize educational programs, skill development and research activities.

Indian Pharmacopoeia (IP)

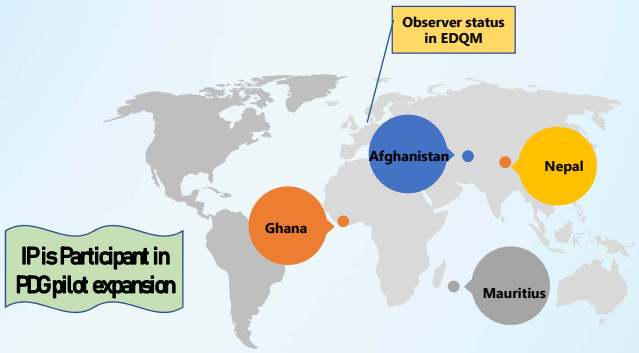
Indian Pharmacopoeia-

- A book of standards published by Indian Pharmacopoeia Commission (IPC). IP specifies the Standards of Quality (identify, purity and strength) of the drugs imported, manufactured for sale, stocked or exhibited for sale or distributed in India.

- Official book of standards for drug included therein, in terms of the Second Schedule to the Drugs and Cosmetics Act, 1940

Journey of IP Editions

Recognition of IP in Foreign Countries

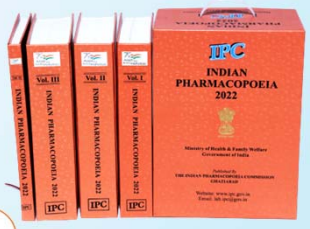


IP has been accepted as a book of standards in a total of **<u>04 countries</u>**

Inclusion & Exclusion Criteria Of Drugs In IP

INCLUSION CRITERIA

- Drugs used in National Health Programs of India
- Drugs listed in Essential Medicines List
- Drugs approved by CDSCO
- Fixed Dose Combinations approved by CDSCO and recommended by the IPC
- Drugs approved by IPC

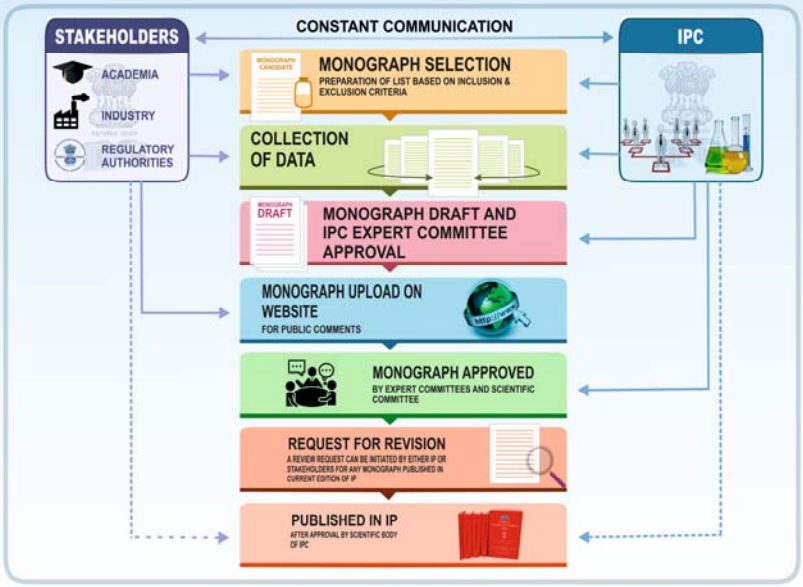


EXCLUSION CRITERIA

- Drugs banned in India
- Obsolete Drugs
- Drugs considered inappropriate by IPC

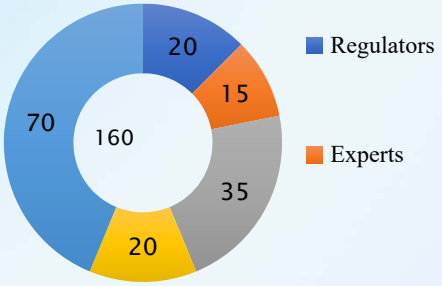
Indian Pharmacopoeia Reference Standards & Impurity Standards | Indian Pharmacopoeia (IP) | National Formulary of India (NFI) | National Coordination Centre-Pharmacovigilance Programme of India

IPC's Monograph Development Process



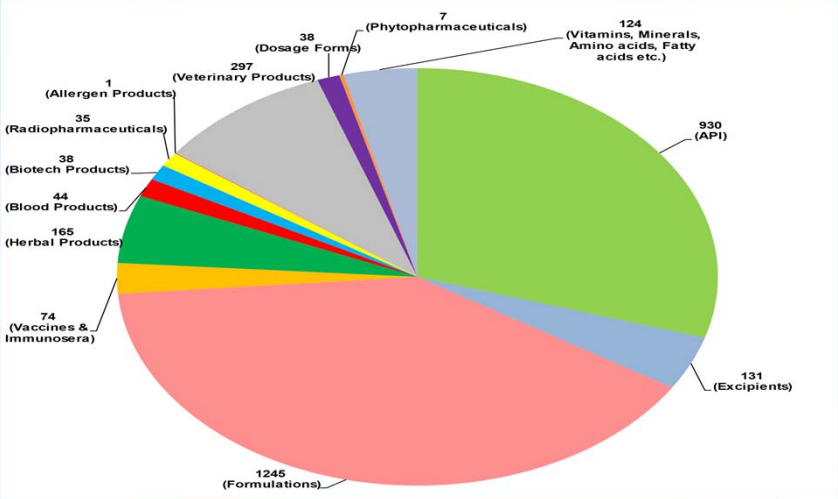
The Experts Behind Our Standards

160 Scientific- Experts- Volunteers and Government Representatives



- **Leaders in their representative fields in industry, academia, healthcare, regulatory**
- **Together they contribute to standards development through consensus-driven decisions achieved through Expert Committees**
- **Regulators and Government laboratories also contribute through suggesting new methodologies and upgrading existing ones.**

IP 2022 : Monograph Status



Total monographs in IP 2022 (9th Edition): 3152

Pyrogenicity test in Indian Pharmacopoeia

IP 1955
- Rabbit Pyrogen test (RPT) Included

IP 1966
- General chapter 2.2.3. Bacterial endotoxins & parenteral preparations

IP 2014
- Provision for Replacement/ Alternate to RPT included

BET
- Vaccines- 10
- rDNA therapeutics-19
- Chemical and Veterinary products-49
- Blood and blood related products-01

BET or RPT:
- Blood and blood related products -09

IP 2018
- General chapter 2.2.25. Monocyte Activation test

BET
- Vaccines- 12
- rDNA therapeutics-35
- Chemical and Veterinary products-85
- Blood and blood related products-01

BET or RPT:
- Vaccines- 17
- Blood and blood related products -09

IP 2022
- General Chapter Guidelines on the Bacterial Endotoxins Tests

BET
- Vaccines- 12
- rDNA therapeutics-38
- Chemical and Veterinary products-344
- Blood and blood related products-02

BET or RPT:
- Vaccines- 24
- Blood and blood related products -09

IP 2022: Pyrogenicity testing status

IP 2022, Biological methods: 2.2.8. Pyrogens

Test animal: Adult rabbit of either sex, Wt. NLT 1.5 kg

Tests: Preliminary test (Sham test), Main test

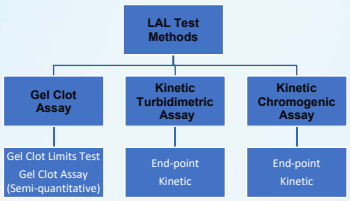
Observation: Raise in body temperature

Only Rabbit pyrogen test (RPT) -06 individual monographs
03 general requirements

RPT or BET- Vaccines for human use
Blood & Blood related products

IP 2022: Pyrogenicity testing status

IP 2022, Biological methods: 2.2.3. Bacterial Endotoxins

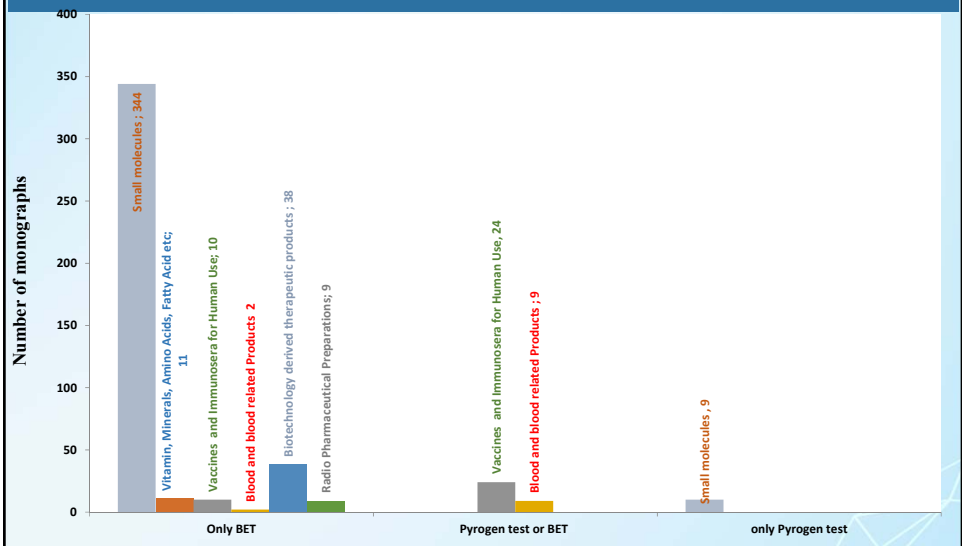


Most parenteral formulation : Bacterial endotoxin test (BET) using LAL reagent

Few Vaccines for human use and Blood and Blood related products: Alternative approach as 'Pyrogen test or BET, if justified and authorized by NRA

A guideline for Alternate test method to Bacterial endotoxin test is uploaded on IPC website for public comment before its inclusion in IP

IP 2022: Pyrogenicity testing status



Indian Pharmacopoeia Reference Standards & Impurity Standards | Indian Pharmacopoeia (IP) | National Formulary of India (NFI) | National Coordination Centre-Pharmacovigilance Programme of India

Monocyte activation Test (MAT)

- General chapter for Monocyte Activation Test was introduced in IP 2018: 2.2.25

- Challenges for adopting Monocyte Activation Test (MAT) faced by stakeholders:
  - Lack of hands on experience and needs training
  - No private or government lab to provide such training
  - Lack of clarity on use for different technology available under MAT
  - Very high cost of available test kit for MAT

Other Proposal from Stakeholder

IPC receives proposal for inclusion of on rFC or rLAL in IP

Justification:

- Sustainable method to replace LAL from horse shoe crab (endagered/vulnerable), Sustainability in terms of horseshoe crab population diminishment and its availability in limited geographical region
- Reproducibility-no lot to lot variability in production of rFC
- Specificity-no Factor G hence no false positive

Challenge:

Performance equivalence/comparision only with LAL test is sufficient or with Rabbit pyrogen test also required?

Alternatives To Animal Methods

IPC has constituted a separate expert working group for 'Alternatives to Animal Methods'

IPC adopts any one or all of the following strategy

- Comparability and applicability of suitable non-animal method/test in place of current *in vivo* method/test
- Alternative approaches based on scientific literature, retrospective data (ex: ATT), GMP and Pharmacovigilance in place etc
- Implementation of consistency approach
- International harmonization of regulatory requirements-WHO TRS and other pharmacopoeia

Conclusion

- IPC is committed in replacing rabbit pyrogen test, where ever possible
- MAT general chapter is included since IP 2018, but challenges faced by stakeholders needs to be rectified
- Guideline for Alternate test method to BET is uploaded on IPC website for public comment
- General notices in IP for alternative methods, stakeholders may adopt alternate method with the approval of regulatory authority
- Other alternate technologies (rLAL, rFc) are under discussion
- Stakeholder may come forward for considering newer technologies during new product development & approval
- Newer technology providers- may adopt cost-effective approach in developing new technologies
  - may focus on new products class, which are under process for regulatory approval
  - may focus on products class where interference are more like blood products, vaccines etc.

Publications

- Shruti Rastogi, **M. Kalaivani**, Amandeep K Bhatia, Jai Prakash, G.N. Singh. Implementing the principle of the 3Rs through the Indian Pharmacopoeia. Therapeutic Innovations and Regulatory Science. DOI: 10.1177/2168479015572371.

Biologics Division team
Indian Pharmacopoeia Commission
Ghaziabad, India

Thank You

USE OF IP & IPRS IS SOCIAL AND LEGAL OBLIGATION FOR "IP" PRODUCTS