This is a transcript of the rapidmicrobiology podcast episode:

**Why MAT will Save Rabbits and Product Recalls**

**Paul (Podcast host):** Hello and welcome to the rapidmicrobiology podcast. And I'm your host, Paul Carton. Today's topic is on pyrogen testing that can be done for the release of safe parenterals, which is of particular interest to SARS-CoV-2 vaccine manufacturers.

We will be putting particular focus on using the monocyte activation test to detect these pyrogens, which can cause severe reactions in the recipient if undetected

Our guest today is Laure Roberts, global product manager at ‎Merck KGaA, Darmstadt, Germany, who helped launch their own monocyte activation test to the market, namely the PyroMAT system. Hello, Laure, and welcome to The Rapidmicrobiology Podcast.

**Laure Robert: (Podcast guest):** Hello, Paul.

**Paul:** Laure, I understand there is a concentration of Pyrogens, which is allowed in a product for release, known as the Contaminant Limit Concentration, or CLC. Could these minute levels of Pyrogens be the cause of side effects stated on parenterals?

**Laure:** Pyrogens, they are fever-inducing agents, types of contaminants that can lead to a fever-reaction in the human body. And the idea with the Pyrogen Test is to check that the amount of Pyrogens in a product is below certain concentration. The CLC, as you just said. And if this amount is below the CLC, actually it means that there is no risk of a Pyrogenic reaction in the body. The risk of a side effect, a fever reaction, is there only if the concentration of Pyrogens in the product is above the CLC that is defined for each product, so each pharmaceutical.

**Paul:** Okay. And where, where would you find the CLC threshold for each product.

**Laure:** It depends, for some products, it can be found in the product monograph in the pharmacopeia. And if the product does not have a monograph in the pharmacopeia, then the CFC can be calculated using a specific formula. That depends on different things. It depends on the route of administration, the dosage, these kind of things. And the end-user can calculate the CLC to be applied for his product.

**Paul:** Okay. Let's talk about the traditional way of detecting pyrogens, and that's known as the rabbit pyrogen test, which is still in use today. Some estimates say there's in the region of 200,000 rabbits being used per year for this pyrogen test. Could I ask you, in this day and age, in your opinion, why are laboratories still performing a rabbit pyrogen test, when a non-animal based Monocyte Activation Test is widely available?

**Laure:** Yes, that's a good question. Actually, the rabbit pyrogen test was the first pyrogen test available. It's kind of the traditional way of testing and most pharma companies which have validated these tests, do not really want to change because of the validation effort that it would represent to change the test method. Usually in the pharmaceutical industry, the main cause for change of test method is a regulatory push. Currently, the regulations are evolving to reduce the use of animal based tests, but still the push from regulatory bodies is not strong enough and is often considered as a recommendation.

Another big issue for the Monocyte Activation Test is the fact that the regulations are very different from one country to another, or at least from an area to another. We see, for example, that it's accepted by regulatory bodies in Europe, whereas in North America, it's still considered as an alternative method, which means that the validation is more complex to perform. That's why currently, even if the Monocyte Activation Test has clear advantages compared to the rabbit pyrogen test, the pharma companies are still at the stage where they evaluate the technology, they show some interest, but they do not put all the efforts and resources for the validation because it's still not considered as mandatory.

**Paul:** Okay. It's just a matter of regulatory agencies not pushing for non-animal based tests and companies not wanting to go through all the validation needed because, the way they see it, both tests are the same in terms of sensitivity, is that right?

**Laure:** No. Actually, the Monocyte Activation Test has really better performances. It's clearly more sensitive, more robust, more standardized. So really a lot of advantages over the rabbit pyrogen test. And actually, it's interesting to see that, for example, in Germany, it's now forbidden to perform the rabbit pyrogen test when there is an individual test that is working for the product. And we see clearly that in Germany, the Monocyte Activation Test adoption is higher than in other countries.

**Paul:** Okay. Germany leading the way in that respect. As you say, there's a geographical disparity in relation to the standardization of this MAT test. Is it now in the international pharmacopeia or if it needs to be validated, how would you do with that?

**Laure:** Yes, it really depends on the geography. For example, in Europe, as I said, the method is really considered as a compendial method in the pharmacopeia. In that case, it's quite straightforward to perform the validation because when the method is considered compendial, you just need to perform what we call a product specific validation. It's a kind of a suitability test to check that the test method is suitable for the product to be tested. But in other geographies, such as North America or most of the Asian countries, the method is still considered as an alternative method. And in that case, it requires a full method validation, which is more complex, and more things to check, several parameters, such as the robustness, the ruggedness, this kind of thing regarding the test method. And this requires more resources.

**Paul:** Okay. Can you just explain for me why a pyrogen test is done instead of a specific endotoxin test and vice- versa~~?~~

**Laure:** It depends. In such cases, it can be a product specific. It's product specific when there is something written in the product monograph. If the product monograph clearly mentions one method or the other as a reference method for the release, then you need to follow this guideline. If the method is not precise in the product monograph, then it's up to the end user to decide which method best suits their needs.

The recommendation currently in such case, is to perform a risk assessment regarding the production process. The idea is really to check if a contamination coming from non- endotoxin pyrogens can be ruled out because the bacterial endotoxin test, which is often called as LAL test as well, is able only to detect endotoxin. If you can prove that in your production process, there is no risk of getting contaminants from other sources than endotoxin, then it would be okay to perform the endotoxin test as a release test. If not, If the risk assessment shows different causes that could lead to a contamination from non-endotoxin pyrogen, then a full pyrogen test should be performed.

**Laure:** So really based on this risk assessment, it's possible to make a decision. But another way of seeing it, a suggestion that I would make, to be on the safe side, it's to use the bacterial endotoxin tests in process. All along the process, to monitor the level of contamination, to see if all along the process, the level of contamination is decreasing and that the process is under control. And finally, for the release of the final product, I would recommend to perform a pyrogen test, a Monocyte Activation Test, really to be sure that the product is safe to use in the human body and that there is no pyrogenicity.

**Paul:** Okay. you do a risk assessment test to see if there's non-endotoxin pyrogens or endotoxins present, and based on those risk assessment results. Let's say if you only found endotoxins, after you perform your LAL endotoxin test for in processbut you advise still do pyrogen test on final release to be sure.

**Laure:** Yes. But again, it's a decision to be made by the end user. But I think it's a good recommendation. Yes.

**Paul:** Okay. And let's get on to the PyroMAT test. There are a number of MAT kit providers on the market, and you'd know this because you've helped launch the PyroMAT test. What separates your PyroMAT test from others on the market?

**Laure:** The main difference is the use of a cell line as a source of monocytes. Basically Monocyte Activation Test uses monocytes for the reaction to reproduce the human immune reaction towards pyrogens. And the different test methods are different because of the source of monocyte that is used. Our competitors are using monocytes from blood. They rely on blood donation and the monocytes are isolated from the blood and they collect what we call PBMC, peripheral blood mononuclear cells. And in our case, we are using a cell line called the Mono Mac 6 cell line, MM6, and this cell line was validated to be used for the Monocyte Activation Tests. And since the launch of the product, we have performed a lot of tests to show that the use of the MM6 cell line is suitable for MAT, including for the detection of non-endotoxin pyrogens.

**Paul:** Okay, there's variability in other tests because they are using human blood, but your MM6 cell lines are synthetic and same throughout. But, can I ask you, is the variability in those human blood donors not an advantage if you're trying to simulate how pyrogens might affect 100 people, for instance?

**Laure:** They are using monocytes that are isolated from the whole blood. What they are doing to mitigate the risk of a variability, is they are pulling blood from different donors, and then they are taking the monocytes, isolating the monocytes, but still from one batch to another, you will get monocytes that come from different donors, which can lead to variability. And it's one of the biggest advantage of using a cell line. What we are doing is to standardize the production process. In the end, you get a standardized reactivity in the Monocyte Activation Test. We are looking at having very standardized reactivity from one batch to another.

**Paul:** Let's move on to quality control for the presence of pyrogens because it's getting more and more complicated as production processes for biotechnology and cell therapy products bring new risks of various contaminants, such as non-endotoxin pyrogens that may enter the final product, like viruses that can come from animal based raw materials or gram-negative bacteria from contamination events. How do you make sure your pyrogen tests keep up with the innovations and potential risks associated with this complex sector?

**Laure:** Yes, you're right. The production processes have evolved a lot in the recent years. It's true that in the past, the main source of contamination in pharmaceutical processes was water. And that's why the main cost for contamination was gram-negative bacteria and it made sense to test for endotoxin only because endotoxin is coming from gram-negative bacteria. But now with this new kind of production processes, we see that there are new biological sources of contamination that are directly entering the production process. And because of this, it's not only endotoxin that can remain in the final product, but also what we call the non-endotoxin pyrogens. And those NEPs, non-endotoxin pyrogens, they need to be identified, or we need at least to detect that they are present in the product. And that's why we need the pyrogen test.

**Laure:** To be sure that our product, the PyroMAT system can detect such kind of contaminants, we have performed a lot of tests to verify the ability of the PyroMAT system to detect the NEPs. We have selected different non- endotoxin pyrogen controls, and we have checked that the Mono Mac 6 cells can actually detect those controls. And to continue with our work, we are now trying to provide additional NEP controls to our customers. So it's something we are working on. The idea is that they can then choose positive control that really reflects the type of contaminants that can be found in that process.

**Paul:** Okay. Essentially you're allowing your customers to spike a sample with a contaminant they're concerned with and run your MAT to see if it can detect it?

**Laure:** Exactly, yes.

**Paul:** Okay. let's say from a practical point of view, what are the steps to successfully implement MAT for pyrogen testing in a QC laboratory, and how can you support customers in this adoption?

**Laure:** The first step, which actually it's not even a first step, but a prerequisite, I would say, for me it's to get trained on the test method. To get trained on Monocyte Activation Test and how to perform it, because MAT is actually quite a manual workflow with some pipetting steps and to ensure consistency in the results and to avoid errors that are linked to operating, I would strongly recommend the operators to get trained before moving to Monocyte Activation Tests.

Once the customers have made their choice and really want to move to MAT for the pyrogen test, I think that the first step is to perform a feasibility test, which means that they would have to check in one single assay.

They would have to check in one single essay that the product they want to test, the pharmaceutical sample, is not interfering with the monocyte activation tests. This is quite an easy test to do. And it's really a first step to see if you can continue in the process.

If the feasibility study is successful, then the user can move to validation. As I said before, depending on the local regulation, the validation can be different. If MAT is considered as a compendial method, they can move directly to what we call product specific validation, which is a reduced validation, an easy one. Which is a suitability test, I would say, to be performed on three batches of the pharmaceutical sample to be tested.

**Laure:** If the method is not compendial in the country and considered as an alternative method, then they need to perform a full method validation to get an approval then from the regulatory body. Once the validation is finished, they can then submit a file to regulatory bodies to show that they want to implement MAT as a release test for quality control.

And once they get an approval, they can move to routine testing. To support our customers in their adoption, we can actually help with these different steps. Mainly for the training, the feasibility and the validation, because we do have quite a wide offer of services that we can provide to customers.

**Paul:** You can provide most of those services to help that long validation happen. Which could be an obstacle for many people adopting MAT. Okay. Thank you, Laure, for a detailed explanation on the monocyte activation test. And I wish you all the best with your PyroMAT test.

**Laure:** Thank you.

**Paul:** Hopefully, it avoids further animal-based testing. And thank you, the listener, for making the time.

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