MONOCYTE-ACTIVATION TEST

The monocyte activation test (MAT) is a test that is based on the reaction of human blood cells [monocytes] to pyrogenic substances. The monocytes while reacting to the pyrogenic substances produce cytokines such as Interleukin [IL – 6], Tumour Necrosis Factor-α [TNFα] etc. This is a part of the fever response in humans. The MAT assay measures the cytokines produced during the reaction with pyrogens and therefore can co-relate to the pyrogenicity of the product. Similarly the temperature increase measured in the Rabbit Pyrogen Test is correlated to the pyrogenicity of the product. Therefore, the MAT test, upon due validation, can be used as a suitable alternate to the Rabbit Pyrogen Test.

In order to use the monocyte activation test as an alternative to rabbit pyrogen test, validation data for the applicability of the MAT to the product is required. Validation data from 3 production batches should include details of sample preparation to adequately address the interfering factors. Additionally, the data from 3 production batches to correlate the rabbit pyrogen test with MAT needs to be provided to establish equivalence.

In the absence of a validated non-endotoxin standard and since the pyrogens group of substances includes bacterial endotoxins, an endotoxin reference standard that has been calibrated against the International Endotoxin Standard could be used for the MAT.

Additionally, as BET specifically detects bacterial endotoxins and a good correlation has been established between the bacterial endotoxins and the rabbit pyrogenic response, we would need to use the bacterial endotoxin standards and follow the related established safety limits such as threshold endotoxin concentration and allowable endotoxin concentration while using the MAT.

The following two methods could be used for carrying out MAT test.

1. Quantitative test
2. Semi-quantitative test

The test should be carried out in a manner that avoids pyrogen contamination.

The maximum valid dilution (MVD) is the maximum allowable dilution of a sample at which the pyrogen limit can be determined. Determine the MVD using the following expression:

\[ \frac{PL \times C}{LOD} \]

\(PL\) = Pyrogen limit ;

\(C\) = Concentration of test solution;

\(LOD\) = Limit of detection.

The acceptance criterion for a pass/fail is the pyrogen limit (PL), which is expressed in endotoxin equivalents per milligram or millilitre, or in units of biological activity of the preparation under examination.

The PL is calculated using the following expression:

\[ \frac{K}{M} \]

\(K\) = threshold pyrogenic dose of endotoxin per kilogram of body mass;

\(M\) = maximum recommended bolus dose of product per kilogram of body mass.

The endotoxin limits depends on the product and its route of administration and is stated in monographs.
Values for K suggested are

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>K (EU of endotoxin per kilogram of body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>5.0</td>
</tr>
<tr>
<td>Intravenous, for radiopharmaceuticals</td>
<td>2.5</td>
</tr>
<tr>
<td>Intrathecal</td>
<td>0.2</td>
</tr>
</tbody>
</table>

For other routes, the acceptance criterion for bacterial endotoxins is generally determined on the basis of results obtained during the development of the preparation.

When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.

Where an endotoxin limit (EL) has been specified for a product, the PL is the same as the EL, unless otherwise prescribed. In this case, the concentration of test solution is expressed in mg/mL if the endotoxin limit is specified by mass (EU/mg), in Units/mL if the endotoxin limit is specified by unit of biological activity (EU/Unit), in mL/mL if the endotoxin limit is specified by volume (EU/mL).

The cut-off value is calculated using the following expression:

$$\bar{\eta} = 3\sigma$$

$\bar{\eta}$ = mean of the 4 replicates for the responses to the blank$A_\omega$;
$\sigma$ = standard deviation of the 4 replicates of the responses to the blank$A_\omega$.

The cut-off value is expressed in units appropriate to the read-out.

The limit of detection (LOD) is determined using the endotoxin standard curve. The LOD is the concentration of endotoxin corresponding to the cut-off value. For the purpose of the test, the LOD is expressed as endotoxin equivalents per millilitre.

**General Procedure**

A test solution of the sample is incubated with a source of human monocytes or human monocyctic cells and the responses viz. levels of cytokines synthesised are compared with responses to standard endotoxin being tested. The chosen read-out method is calibrated using the appropriate standard.

The source of human monocytes or human monocyctic cells can be from human heparinised peripheral blood that is not more than 4 hour old, or human peripheral blood mononuclear cells (PBMC) isolated, and diluted with appropriate medium or saline to 2-50 per cent v/v (final concentration) so as to achieve a final cell density of 0.1-1.0 x $10^6$ cells per receptacle.

**Cell Sources and Qualification**

**Whole Blood**

The whole blood needs to be obtained from single donor or from pooled whole blood. It should be in line with the requirements described under Qualification of blood donors and Qualification of cells pooled from a number of donors respectively.

**Peripheral Blood Mononuclear Cells (PBMC)**

PBMC are isolated from blood obtained from single donors or from pooled whole blood. The cells should be in line with the requirements described under Qualification of blood donors and Qualification of cells pooled from a number of donors respectively.

**Qualification of Blood Donors**

Blood donors are to satisfy the following qualification criteria:
1. Donor should be in good health.
2. Donor should not be suffering from any bacterial or viral infections.
3. Donor should be free from the symptoms of any infection for a period of at least one week prior to the donation of blood.

4. Donor is not to have taken non-steroidal anti-inflammatory drugs during the 48 hour prior to donating blood.

5. Donor is not to have taken non-steroidal anti-inflammatory drugs during the 7 days prior to donating blood.

Following cases would not qualify as blood donors:

i. Individuals who have been administered immune-suppressants.

ii. Individuals who have been administered drugs known to influence the production of the chosen readout.

Qualification of Cells Pooled From a Number of Donors

Pools (of whole blood or blood fractions, e.g., PBMC), must be from a minimum of 4 individual donors, preferably equal volumes of blood or cells from each of them. For the qualification of pooled cells, within 4 hours of collection of blood, a dose-response curve should be generated using standard endotoxin concentrations.

Preparatory Testing

The manufacturer’s instructions of the MAT kits need to be followed to establish the appropriate standard curve, determine the non-interfering product concentration so as to ensure detection of the pyrogens.

Test for Interfering Factors

Initial test must be performed to ensure that the test solution does not have interfering factors. It is necessary to re-validate the test method when any changes are effected to the test procedure that are likely to influence the result of the test. Prepare dilutions not exceeding MVD of the product and spike with endotoxin concentration near or equal to the mid-point of the standard curve or equal to twice the LOD. The mean recovery needs to be calculated by subtracting the mean concentration of endotoxin equivalents in the solution (if any) from that in the spiked solution. The test solution is considered free of interfering factors if the recovery of endotoxin is within 50 to 200 per cent of the spiked endotoxin concentration.

**Interpretation of the test results:**

The test samples complies with the test when:

1. All negative controls are negative
2. Spike recovery is between 50 and 200 per cent.
3. The pyrogen concentration of the test solution is less than the PL.