Ryanodine test - optional and standardized for the EMHG

**Ryanodine**
High purity (Calbiochem).

**Stock solution**
100 micromol litre\(^{-1}\) in distilled water.

**Storage**
Refrigerator at 4°C.

**Test concentration**
Final bath concentration of 1 micromol litre\(^{-1}\).

**General guidelines**
The ryanodine test is performed similarly to other contracture tests described in the EMHG protocol:
1. A fresh muscle specimen must be used.
2. The specimen is electrically stimulated with a 1 ms supra-maximal stimulus at a frequency of 0.2 Hz.
3. The test is performed at optimal length (L\(_o\)).
4. Baseline must not vary more than 2 mN (0.2 g) within a 10 min period before the addition of ryanodine.
5. A single bolus dose technique is used to reach a final bath concentration of 1 micromol litre\(^{-1}\). If the tissue bath is continuously perfused with Krebs solution, either the perfusion is stopped or a Krebs-ryanodine solution is perfused. The final bath concentration of ryanodine must be reached **within 1 min**.

**Test parameters**
1. Similar to other tests: weight (mg) and length (mm) of specimen, twitch height (mN), maximum preload (mN), predrug baseline (mN), lowpoint baseline (mN).
2. Specific parameters: onset time (OT) of contracture (min), time for development of a 10 mN (1 g) contracture (10T (min)), contracture velocity (dT = 10T-OT) (min), and optionally: time for development of contracture maximum (Tmax) (min), maximum contracture (mN).

**Quality control**
The concentration of ryanodine in the tissue bath must be periodically checked by HPLC.

![Diagram of muscle contraction](attachment:image.png)

In this example, predrug baseline = low point baseline.