Determination of biological activity of lectin in Suilectin preparation

I. Determination of biological activity of lectin in Suilectin preparation

1. Scope

This procedure specifies the determination of lectin activity on the base of their haemagglutinating activity. The method is suitable and validated exclusively for the determination of lectin activity and exclusively in Suilectin preparation.

2. Terms and definitions

For the purpose of this procedure, following terms and definitions apply.

2.1 Haemagglutination activity unit HAU

One haemagglutination unit expresses such propriety of the lectin preparation which solution at the concentration 1 mg/ml causes haemagglutination in at least 50% of rat erythrocytes under conditions specified in this procedure.

3. Principle

Lectins extracted from Suilectin preparation agglutinate rat erythrocytes for 30 min to 1 hour at room temperature. Agglutination of rat erythrocytes is observed under a microscope. Quantity of haemagglutination units (HAU) of 1 mg of a tested lectin preparation is defined as the reciprocal of the concentration of solution (mg/ml) in a subsequent dilution, at which there is still haemagglutination observed in at least 50% of rat erythrocytes.

4. Reagents

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade and distilled or demineralized water of equivalent purity.

WARNING – This procedure requires handling of hazardous substances. Apply local regulations for potentially hazardous biochemicals to minimize risks to organizational, technical and personal safety.

4.1 Physiological saline (NaCl) – 0.9% solution

4.2 Heparin

4.3 Rat erythrocytes (see clause 6.1)

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5. Apparatus

Usual laboratory apparatus, in particular, the following.

5.1 Analytical balance, capable of being read to at least 0.1 mg

5.2 Centrifuge, capable of 2000 rpm

5.3 Microscope, 250 x

6. Procedure

6.1 Erythrocyte preparation

1 ml of blood collected from a Wistar rat (with one drop of heparin) is diluted 1:20 in an isotonic salt solution (0.9% NaCl). The blood is then washed by centrifugation (2000 rpm, 15 min, +4°C), the supernatant discarded and resuspended in the original volume with 0.9% saline. This procedure is repeated three times. Afterwards 2% erythrocyte solution is prepared (dilution 1:50).

6.2 Suilectin preparation

200 mg of the powder preparation is taken for analysis, the preparation is diluted in 8 ml of isotonic salt solution. The final concentration – 25 mg/ml.

A series of double-fold increasing diluted solution is prepared:

Dilution: 1:2 ; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256; 1:512

6.3 Assay execution

The test is done simultaneously in Ependorf test tubes and standard microscope glass (not degreased).

The reactionary mixture in Ependorf test tubes consists of:

100 µl of isotonic salt solution

100 µl of 2% erythrocyte

100 µl of examined solution (relevant dilution)

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After thorough mixing of the components, 50 µl is collected from each test tube, put on microscope glass, and incubated in humid chamber, at room temperature for 30 minutes up to an hour (it is essential to pay attention for the drops not to dry out). The rest of the preparation is incubated in capped Ependorf test tubes (for no less than an hour, prolonging the period does not influence the result). After the incubation, Ependorf test tubes' content is stirred with pipette to remove the residue from the bottom and walls of the test tube, afterwards 50 µl is collected and placed on microscope glass for further microscope analysis.

For comparison purposes the test is done with negative control:

200 µl of isotonic salt solution

100 µl of 2% erythrocyte

The result is read with the use of microscope with 250x magnification. Signs: +, +/-, -, are used to record the result, whereas „+" means the presence of erythrocytes haemagglutination and „–„ its absence.

For convenience sake it is possible to omit the incubation at microscope glass, fair enough results are obtained by incubating the preparation in the capped Ependorf test tubes.

6.4 Defining the number of haemagglutinating units in the preparation

One haemagglutination unit (1 HAU) of activity is defined as the amount of 1 mg of lectin preparation/ml in the last dilution giving at least 50% agglutination of rat erythrocytes, according to the above described haemagglutination assay. The activity of the preparation is 1 HAU/mg.

Number of haemagglutinating units (HAU) of 1 mg of the lectin preparation is described as the reverse preparation concentration (in mg/ml) in the next dilution, when at least 50% agglutination of rat erythrocytes is observed.

For Suilectin preparation the activity in haemagglutinating units is usually stated for 100 mg of the preparation.

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Example:

The above described test was used for double haemagglutination activity study of archive Suilectin sample. In both cases the activity was 128 HU/100mg.

	200mg / 8ml = 25 mg/ml								
1	2	3	4	5	6	7	8	9	Sample number
25.00	12.50	6.25	3.12	1.5625	0.7813	0.3906	0.1953	0.0977	mg/ml
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	dilution
++	++	++	++	++	1.28	±	-	-	HU/mg of preparation according to the Swedish definition

7. Performance characteristics

Limit of quantification is equal to 4 haemagglutinating units (HAU) / 100 g of Suilectin.

8. Test report

Following information is to be included in the test report:

- all information necessary for identification of the sample tested
- a reference to this method
- the result
- any deviations from the procedure specified
- any anomalies observed during the rest
- the date of the test