RABBIT BLOOD SUGAR METHOD—QUANTITATIVE

be collected effectively from the central auricular artery.

The Rabbit Blood Sugar Method—Quantitative is used to determine the specific activities of insulin analogs. To mine the potency of Insulin Reference Standards, for the validation of the stability of new insulin preparations, and to determine the activities of insulin analogs.

ASSAY

RABBIT BLOOD SUGAR METHOD—QUANTITATIVE

Diluent: Prepare an aqueous solution containing 0.1%–0.25% (w/v) of either cresol or phenol, 1.4%–1.8% (w/v) of glycerin, and sufficient hydrochloric acid to produce a pH between 2.5 and 3.5, unless otherwise directed in the individual monograph.

Standard stock solution: Prepare a solution containing 40 USP Insulin Units/mL of USP Insulin RS of the appropriate species in Diluent and having a pH between 2.5 and 3.5, unless otherwise directed in the individual monograph. For insulin of mixed bovine and porcine species, prepare a solution containing 34.8 USP Insulin Beef Units/mL and 5.2 USP Insulin Pork Units/mL in Diluent and having a pH between 2.5 and 3.5.

Sample stock solution: Proceed as directed in the Standard stock solution, except to use a suitable quantity of the preparation under test in place of USP Insulin RS of the appropriate species. The Sample stock solution contains about 40 USP Insulin Units/mL.

Sample solutions: Dilute portions of the Sample stock solution with Diluent to make two dilutions, one to contain 1.0 USP Insulin Unit/mL (Standard solution 1), and the other to contain 2.0 USP Insulin Units/mL (Standard solution 2).

Sample preparations: Pipet into separate, suitable vessels 0.1 mL of each Blood sample and 0.9 mL of Anticoagulant solution.

Blood samples: At 1 h ± 5 min and 2 1/2 h ± 5 min after the time of injection, obtain from each rabbit a suitable blood specimen from a marginal ear vein. Blood can also be collected effectively from the central auricular artery.

Anticoagulant solution: Dissolve 1 g of edetate sodium and 200 mg of sodium fluoride in 1 L of water, and mix.

Dextrose determination: Determine the dextrose content of the blood specimens by a suitable procedure that is adapted to automated analysis. The following procedure may be used.

Dextrose standard preparations: Transfer known concentrations of USP Dextrose RS to suitable vessels, and dilute quantitatively and stepwise with Anticoagulant solution (1:9) to obtain a range of dextrose concentrations in the rabbit blood samples.

Calculation: Calculate the response of each rabbit to each injection from the sum of the two blood sugar values, and compute:

\[ T_4 = T_1 + T_2 + T_3 - T_4 \]

Blood and dextrose determinations are similarly determined at the start and the end of each run.

Calculation: Calculate the response of each rabbit to each injection from the sum of the two blood sugar values, and subtract its response, disregarding the chronological order in which the responses were observed, to obtain the individual differences, \( y \), as shown in Table 2.

When the data for one or more rabbits are missing in an assay, do not use the confidence interval formulas given here, but seek statistical help. The data can still be analyzed with proper analysis of variance.

Calculation: Calculate the response of each rabbit to each injection from the sum of the two blood sugar values, and subtract its response, disregarding the chronological order in which the responses were observed, to obtain the individual differences, \( y \), as shown in Table 2.

When the data for one or more rabbits are missing in an assay, do not use the confidence interval formulas given here, but seek statistical help. The data can still be analyzed with proper analysis of variance.
and

\[ T_0 = T_1 + T_2 + T_3 + T_4 \]

The logarithm of the relative potency of the test dilutions is

\[ M' = 0.301 T_0 / T_b \]

The potency of the injection in USP Units/mg equals the antilog (log \( R + M' \)), where:

\[ R = v_S / v_U \]

\[ v_S = \text{number of USP Units/mL of the Standard solution} \]

\[ v_U = \text{number of mg/mL of insulin of the corresponding Sample solution} \]

Determine the 95% confidence interval for the log-relative potency using Fieller's Theorem (see Appendix and Design and Analysis of Biological Assays (111)). If the confidence interval is more than 0.082, which corresponds at \( P = 0.95 \) to confidence limits of about \pm 10\% of the computed potency, repeat the assay until the combined data of the two or more assays, redetermined as described in Combination of Independent Assays in (111), meet this acceptable limit.

<table>
<thead>
<tr>
<th>Group</th>
<th>Differences</th>
<th>Individual Response (( y ))</th>
<th>Total Response (( T ))</th>
<th>Standard Deviations of Differences (( S ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard solution 2 – Sample solution 1</td>
<td>( y_1 )</td>
<td>( T_1 )</td>
<td>( S_1 )</td>
</tr>
<tr>
<td>2</td>
<td>Sample solution 2 – Standard solution 1</td>
<td>( y_2 )</td>
<td>( T_2 )</td>
<td>( S_2 )</td>
</tr>
<tr>
<td>3</td>
<td>Sample solution 2 – Standard solution 1</td>
<td>( y_3 )</td>
<td>( T_3 )</td>
<td>( S_3 )</td>
</tr>
<tr>
<td>4</td>
<td>Standard solution 2 – Sample solution 1</td>
<td>( y_4 )</td>
<td>( T_4 )</td>
<td>( S_4 )</td>
</tr>
</tbody>
</table>

Appendix: Fieller’s Theorem for Determining the Confidence Interval for a Ratio

This version of Fieller’s Theorem is for the case where the numerator and denominator are uncorrelated. The equation assumes that the numerator and denominator are normally distributed and that the groups of rabbits are of equal sizes.

Then, the 95% confidence interval for the ratio is:

\[ M' \pm \frac{t}{T_0} \sqrt{(1 - g) \left( S_0^2 + (M')^2 S_0^2 \right)} \]

where \( t \) (degrees of freedom in the standard errors) = 4(\( k - 1 \)), where \( k \) is the number of rabbits in a group, \( t \) is the upper 97.5 percentile of the \( t \)-distribution with \( f \) degrees of freedom, and

\[ g = \frac{t^2 S_0^2}{T_0^2} \]

If \( g \geq 1 \), the denominator is not significantly different from 0 and the formula does not work.

\[ S_u = 0.301 \sqrt{k} \sqrt{S_1^2 + S_2^2 + S_3^2 + S_4^2} \]

\[ S_d = \sqrt{k} \sqrt{S_1^2 + S_2^2 + S_3^2 + S_4^2} \]

• **Bioidentity Test**

Proceed as directed in Rabbit Blood Sugar Method—Quantitative with the following modifications.

Procedure: Divide the rabbits into four equal groups of two rabbits each.

Calculation: Proceed as directed for Calculation in Rabbit Blood Sugar Method—Quantitative, but do not determine the confidence interval of the log-relative potency, \( M' \).

Interpretation: If the potency value obtained is NLT 15 USP Units/mg, the Bioidentity Test requirement is met. If the potency value is less than 15 USP Units/mg, repeat the test using eight more rabbits. If the average potency of the two sets of tests is NLT 15 USP Units/mg, the requirement of the test is met.

**ADDITIONAL REQUIREMENTS**

**Change to read:**

• **USP Reference Standards** (11)

USP Dextrose RS

• USP Insulin Aspart RS (IRA 1-Mar-2015)

USP Insulin Beef RS

• USP Insulin Glargine RS (IRA 1-Mar-2015)

USP Insulin Human RS

• USP Insulin Lispro RS (IRA 1-Mar-2015)

USP Insulin Pork RS