Comparison of Chicken Plasma and Guinea Pig Serum in a Quantitative Microtiter Method of Determining Microbial Complement Resistance

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SUMMARY. A quantitative microtiter method using chicken plasma is described for determining the degree of complement resistance or sensitivity of avian Escherichia coli isolates. Results obtained with the microtiter method using chicken plasma were compared with results obtained using commercially available standardized guinea pig serum as the source of complement. The test organisms consisted of five isolates of E. coli isolated from chickens. Three isolates were from flocks with colisepticemia; one was from a flock with omphalitis; and one isolate was a non-pathogenic control.

Data were accumulated from the five avian E. coli isolates incubated at 35°C with either chicken plasma or guinea pig serum and with heat-inactivated chicken plasma or guinea pig serum. The microtiter results of the chicken plasma and guinea pig serum had a statistically positive correlation. The use of commercially available guinea pig serum in the test system will allow for standardization of this method.

RESUMEN. Comparación del uso de plasma de pollo con el uso de suero de cobayo en el método de microtitulación cuantitativa para la determinación de la resistencia microbiana al complemento.

Se describe un método de microtitulación cuantitativa que utiliza plasma de pollo para la determinación del grado de resistencia o de sensibilidad al complemento por parte de cepas aviares de Escherichia coli. Los resultados obtenidos mediante el método de microtitulación utilizando plasma de pollo fueron comparados con los resultados obtenidos mediante la utilización de suero comercial estandarizado de cobayo como fuente de complemento disponible. Los microorganismos involucrados en la prueba consistieron en cinco cepas de E. coli provinientes de aves. Tres de las cepas provinieron de parvadas con colisepticemia, una de ellas fue obtenida de una parvada con problemas de omfalitis y otra más era un control no patógeno.

Se obtuvieron datos de las cinco cepas aviares de E. coli incubadas a 35°C ya sea con plasma de pollo o con suero de cobayo y con suero de pollo o de cobayo inactivado con calor. Los resultados de la microtitulación con el plasma de pollo y con el suero de cobayo presentaron una correlación positiva. El uso de suero comercial de cobayo en este sistema permitirá la estandarización de este método.

The in vitro testing of a microorganism’s ability to resist the action of complement is important in the study of disease pathogenesis. At present, four methods are used to determine resistance or sensitivity of microorganisms to the action of complement. These methods include a qualitative microtiter technique (4), a plate-count technique (5,6), an automated system (1,2), and a quantitative microtiter method (3). The microtiter method is non-quantitative; the plate-count technique is time-consuming and labor-intensive; the automated system is expensive; and the quantitative microtiter method utilizes species-specific plasma that interferes with the standardization of the test and comparisons of successive results.

The purpose of the present study was to describe a means of standardizing the quantitative microtiter method using a commercially avail-
able guinea pig serum to study complement resistance. Statistical methods used to classify the microorganisms studied also are described.

MATERIALS AND METHODS

Test organisms. Five avian E. coli isolates were studied: three (Virulent-1, Virulent-2, Virulent-3) were cultured from necropsy samples of birds with colisepticaemia; one (Omphalitis) was from a flock with omphalitis; and one (Avirulent) was from a non-pathogenic control. The isolates had previously been classified as either resistant (R) (isolates from flocks with colisepticaemia), intermediate (I) (isolate from flock with omphalitis), or sensitive (S) (non-pathogenic control) to the action of complement using described methodology (3).

Media. Bacterial isolates were maintained on MacConkey agar plates (Difco Laboratories, Detroit, Mich.). Bacterial isolates used in the microtiter test method were cultured in Luria Bertani (LB) broth (5) and in PG broth (1% peptone plus 1% glucose) (4).

Bacterial resistance to chicken plasma or guinea pig serum by the microtiter method. Two-hour cultures grown in LB broth were diluted 1:50 in PG broth. Then 100 µl (10⁴ colony-forming units) samples were inoculated into flat-bottomed 96-well microtiter plates (Microtest III, Becton-Dickinson Co., Lincoln Park, N.J.). One hundred µl of chicken plasma and heat-inactivated plasma in one set of microtiter plates and a similar quantity of guinea pig serum and heated guinea pig serum added to another set of microtiter plates was diluted (twofold dilutions) from 50% to 0.8%. A medium and culture control also were included. Plates were monitored for growth by an enzyme-linked immunosorbent assay (ELISA) reader (Titertek Multiskan, Flow Laboratories, McLean, Va.) at 492 nm, set in the absorbance mode, using the range of 1.0. Plates were incubated at 35 C and read hourly from 0 to 6 hours (3).

Table 1. Comparison of the mean optical-density values of five isolates of avian Escherichia coli incubated with 12.5% guinea pig serum (complement or heated) and chicken plasma (complement or heated) for 6 hours.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Guinea pig serum</th>
<th>Chicken plasma</th>
<th>Complement group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complement</td>
<td>Heated</td>
<td>Complement</td>
</tr>
<tr>
<td>Virulent-1</td>
<td>0.1634⁺</td>
<td>0.1779⁺</td>
<td>0.2286⁺</td>
</tr>
<tr>
<td>Virulent-2</td>
<td>0.1110⁺</td>
<td>0.1130⁺</td>
<td>0.2540⁺</td>
</tr>
<tr>
<td>Virulent-3</td>
<td>0.1499⁻</td>
<td>0.1597⁻</td>
<td>0.2413⁻</td>
</tr>
<tr>
<td>Omphalitis</td>
<td>0.1167⁺</td>
<td>0.1556⁺</td>
<td>0.2124⁺</td>
</tr>
<tr>
<td>Avirulent</td>
<td>0.0163⁺</td>
<td>0.1909⁻</td>
<td>0.1263⁺</td>
</tr>
</tbody>
</table>

⁺Within a row, values followed by different lower-case superscripts are significantly different.

R = resistant, I = intermediate, S = sensitive.

Sources of chicken plasma and guinea pig serum. Chicken plasma was obtained by wing-bleeding 20 seven-week-old white leghorn chickens. Heparin was used as an anticoagulant. Erythrocytes were removed by centrifugation, and plasma was stored at -70 C.

Guinea pig serum was purchased commercially (ICN ImmunoBiologics, Costa Mesa, Calif.). Serum was derived from pooled samples and assayed by the company for complement activity (positive hemolysis at 0.075 ml at 1:10 dilution).

Statistical analyses. Optical-density data for each isolate of E. coli were subjected to an analysis of variance with a follow-up Tukey test when necessary. For each strain of E. coli, the relationship between guinea pig serum and chicken plasma was examined by generating a correlation matrix using the Pearson product-moment correlation technique. Coefficients of determination ($r^2 \times 100$) also were calculated (7).

For each organism, the optical-density readings representing bacterial growth in the serum or plasma variable were regressed on hours of incubation using an analysis of variance for linear regression (7).

Growth in guinea pig serum and growth in chicken plasma were compared directly by regressing the mean optical-density values of the growth of all of the E. coli strains in guinea pig serum on the mean optical-density values for the same organisms grown in chicken plasma. Correlation coefficients and coefficients of determination were calculated (7).

RESULTS

There were no significant differences in the mean optical-density readings of the five isolates of E. coli when comparisons were made of their growth in chicken plasma or guinea pig serum (complement) and in the heat-inactivated chicken plasma or heat-inactivated guinea pig serum (heat-inactivated complement) (Ta-
ble 1). Isolates found to be resistant, intermediate, or sensitive to the action of complement by use of the chicken plasma system were found to have identical classifications when tested in the guinea pig system (Table 1).

The intercorrelational matrices of each E. coli isolate incubated in each serum variable (chicken plasma, heat-inactivated chicken plasma, guinea pig serum, and heat-inactivated guinea pig serum) were calculated. The intercorrelations were significant ($P < 0.01$) for isolates Virulent-1, Virulent-2, Virulent-3, and Omphalitis when grown in either the chicken plasma or guinea pig serum variables; the intercorrelations ranged from 0.939 to 0.991 (Fig. 1). The intercorrelational matrix for the Avirulent isolate was 0.959 in the heat-inactivated products but 0.621 when comparing the chicken plasma and guinea pig serum. This observation is explained by the fact that the guinea pig serum suppressed bacterial growth completely, while the chicken plasma allowed for slight bacterial growth (Fig. 2).

Bacterial growth in guinea pig serum regressed in a significantly ($P < 0.001$) linear fashion on growth in chicken plasma (Fig. 3). The correlational coefficient ($r$) was 0.815, and the coefficient of determination ($r^2 \times 100$) was 66.4%. Comparison of the growth curves of the five isolates incubated in increasing twofold dilutions of guinea pig serum from 0.8% to 50% indicated that dilutions of 12.5% or 25% were most discriminating.

**DISCUSSION**

The quantitative microtiter method compares favorably with other systems in its ability to classify E. coli as either sensitive, intermediate, or resistant to the action of complement (3). Because the microtiter method is simple and inexpensive, this technique is preferable as a means to quantitate E. coli complement resistance/sensitivity.

A major factor limiting the usefulness of the quantitative microtiter method is that interpretation of future results will depend upon the...
source of chicken plasma (complement). This lack of standardization in the source of complement prompted our tests of the use of standardized commercially available guinea pig serum in the test system.

The positive correlation coefficients of 0.933 or greater reflect an extraordinary direct relationship between the use of chicken plasma and guinea pig serum in the test system. When the guinea pig serum and chicken plasma were compared directly by regressing bacterial growth of the organisms grown in guinea pig serum to bacterial growth in chicken plasma, the statistical differences were highly significant ($P < 0.001$), providing further evidence of this direct relationship.

Statistical comparisons of data generated in the present study indicate that when an E. coli isolate was classified as resistant, intermediate, or sensitive to the action of complement utilizing a chicken plasma system, it was classified similarly when reacted with guinea pig complement. Therefore, the use of guinea pig serum in the microtiter system has the advantages of product availability, standardization of the test at 12.5% or 25% serum, and the ability to compare isolates examined at various times.

REFERENCES


