Plating Efficiency for Primary Hamster Embryo Cells as an Index of Efficacy of Fetal Bovine Serum for Cell Culture

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Attachment and growth of mammalian cells plated at low cell density require optimum conditions for the cells to form colonies. Reliability, reproducibility, and validity of the plating efficiency test for evaluating cell culture sera were determined by measuring the plating efficiency of 37 lots of fetal bovine serum obtained from 8 suppliers (5 lots from each of 7, 2 lots from 1 supplier), by using hamster embryo fibroblasts plated at low cell density. The test revealed considerable variation between lots of serum and between suppliers. The five lots from some suppliers had consistently high plating efficiencies, whereas one or more lots from other suppliers had quite low efficiencies. The results were reproducible in repeated tests, and control experiments indicated that the test measured the efficiency of the test serum independently of the efficiency of the serum used for the primary outgrowth of the hamster embryo cells.

Ability to support attachment and growth of cells under conditions of extremely low cell density is one of the most stringent criteria of the quality of a cell culture serum (4). Many experiments require plating cells so that individual colonies can develop. In vitro testing of chemicals for carcinogenicity, for example, requires making colony counts to evaluate cytotoxicity of the chemical and to score colonies for chemical transformation (3). Hamster embryo cells (HEC) have been used extensively for such studies. In other instances it is desirable to make the most economical use of cells available, and therefore it is advantageous to use a serum that promotes a high plating efficiency.

Our laboratory uses HEC for in vitro carcinogenicity and toxicity studies, in which the HEC usually have a 10 to 18% absolute plating efficiency. For some time we have used the plating efficiency test as a rapid method for selecting fetal bovine sera that could be used for these experiments. To put the test on a more sound basis, we obtained data in a controlled fashion to assess the reliability, reproducibility, and validity of the test. These data were obtained by comparing several lots of serum from each of eight suppliers for the ability to support attachment and growth of cells when only about 1,000 HEC were plated in a 60-mm dish. We found considerable variation between suppliers and between lots of serum from some of the suppliers. These differences were consistent in repeated experiments, and control experiments indicated that the test measured the efficacy of the test serum independently of the serum used for primary outgrowth. Because of the wide interest in methods for evaluating sera and the rapidity of this test, we report our findings here. (This paper was presented at the 24th Annual Meeting of the Tissue Culture Association, Boston, Mass., 5 June 1973.)

MATERIALS AND METHODS

Cell cultures and determination of plating efficiencies. Pregnant Syrian hamsters (strain LVG: LAK) were obtained from the "trailer" colony of Lakeview Hamster Colony, N.J. After 14 to 15 days of gestation, the mother was anesthetized with ether and the embryos were removed aseptically. The embryos were dissected free of maternal and fetal membranes, decapitated and eviscerated, minced, trypsinized, and seeded at 5 x 10⁶ cells per dish into 60- by 15-mm Lux plastic petri dishes. By seeding at such high cell density, the cells were limited in the number of divisions they could undergo.

To determine absolute plating efficiency, cells were trypsinized from the primary cultures, counted, and diluted so as to plate 1,000 cells per 60- by 15-mm Lux plastic petri dish, in 3 ml of medium containing the test serum at 10% concentration. Five replicate dishes were used for each test serum. After 4 days, another 3
ml of the same medium was added. At 9 days, the cultures were rinsed, fixed with buffered Formalin, and stained with Giemsa stain, and the colonies were counted.

Medium. For all experiments reported here, the medium used was BioLabs' modification of Dulbecco's modification of Eagle medium (2). Sodium bicarbonate levels were 1.1 g/liter in the initial plating medium and 2.2 g/liter in the medium added at 4 days. All cells were incubated at 37 C in a 5% CO₂ atmosphere.

Sera. Test sera were ordered from each of eight different suppliers (designated A through H herein) as follows: “Two 100 ml bottles of each of 5 lots of bovine fetal serum” with the appropriate catalogue number. The purchase order did not specify how the supplier was to select the lots to fill the order; it is, of course, possible that one or more suppliers sensed that a survey was being conducted and sent selected sera, but this seems unlikely. One company supplied only 2 lots; therefore, a total of 37 lots was tested. Each serum sample was stored at -20 C. Immediately prior to use, the samples were thawed, heat inactivated (56 C, 30 min), and coded. The data were “decoded” after the tests were completed.

RESULTS

Plating efficiencies of different sera. The 37 lots of serum were tested in two separate experiments (Table 1). The average number of colonies in the second experiment was approximately twice as high as in the first experiment; the reason for the difference is unknown but probably can be accounted for as error in counting the trypsinized primary cells, resulting in different numbers of cells plated. Valid comparisons can be made between lots within each experiment, since all test plates were set up simultaneously and randomly from the same diluted cell suspension.

Absolute plating efficiencies varied from 9.7 and 16.8% for a highly efficient serum (F-1) to 0.2 and 0.6% (B-5) for a serum of low efficiency. The five lots from the two sources (E and F) with the highest efficiencies varied little between lots. There was large lot-to-lot variation from sources B and D. Others, for example A, had less lot-to-lot variation, and all lots of that source were about “average.”

These data were analyzed by analysis of variance to obtain objective, statistical inferences. In both experiments, the variation from dish to dish within the group tested for each lot was quite small; the variation from company to company was far greater than the variation within the five test dishes per lot (P < 0.005) and also greater than the variation between lots from each company (P < 0.025). Taken together, variation between all lots was significantly greater (P < 0.005) than the variation within the test dishes of single lots. (The data of the two lots from company H were omitted to simplify the analysis of variance; inspection of the data indicates this should not change the above statistical inferences.)

To compare the two experiments, the averages of the five lots of serum from each company were normalized by expressing each as a percentage of company F. The companies were then ranked in decreasing order (Table 2). The rankings were quite similar in the two experiments; company D shifted down three ranks and company H shifted down one rank in the second trial. In both instances, however, the differences in the percentages of the companies involved in the shift were quite small; therefore, within experimental error the two experiments gave the same rankings.

Influence of serum used for primary cultures. Because of the use of one serum throughout for the growth of the primary cultures, it is possible that the data could be biased in favor of that supplier. (The serum used for the primary growth of cells in the experiments described in the previous section was from a sixth lot from company F, used for routine purposes in our laboratory.) The most bias could arise between companies E and F, which had the highest, and not very different, plating efficiencies. A given serum, for instance, could select for a certain population of cells that grow
Table 2. Ranking of suppliers of fetal bovine serum by relative plating efficiencies

| Co.* | RPE | Co. | RPE |
|------|-----|-----|-----|
| F    | 100.0 | F   | 100.0 |
| E    | 86.6  | E   | 70.3  |
| D    | 48.2  | A   | 52.9  |
| A    | 43.8  | C   | 51.2  |
| C    | 39.2  | D   | 49.6  |
| G    | 38.9  | G   | 45.5  |
| H    | 37.4  | B   | 34.8  |
| B    | 37.0  | H   | 34.6  |

* Companies are listed in decreasing order.
* RPE, Relative plating efficiency.

Better in that serum; if kept in the same serum for determining plating efficiency, a higher number of colonies might result.

To determine whether the ranking of these two companies could be reversed if a different serum were used for the primary cell cultures, the following experiment was done. HEC were prepared by using serum lots F4, F5, E2, and E1; these were selected from the “high” and the “low” efficiency serum lots of each of companies E and F. Cells grown originally in each of these four lots of serum were then plated in all five lots from each of companies E and F to determine plating efficiencies as before (Table 3). Regardless of the serum used for the primary cell cultures, all lots of serum from company E had absolute plating efficiencies about 70% of that of company F.

It was also important to determine whether the use of a serum with very low plating efficiency for the primary outgrowth would influence the efficiency of the test serum. To test this, cells were initially plated in sera with high, medium, and low efficiencies as determined in the previous test. Cells from each were then plated in sera of high, medium, and low efficiencies (Table 4). The results indicate again that the number of colonies obtained is independent of the efficiency of the serum used for the primary outgrowth.

Discussion

The results of the experiments reported here indicate that sera from different suppliers vary with respect to efficacy in supporting attachment and growth of fastidious cells under the stringent conditions of plating at very low cell densities. These differences are detectable by the test described, which is rapid and reproducible and is a valid measure of the efficacy of the test serum, being independent of the serum used for the primary growth of the cells.

Previously, Boone et al. (1) reported a somewhat similar type of study, except that many more cells were plated (the cell densities they used were about 250 and 500 times those used in the study reported here). At the higher cell densities, variations between suppliers were no greater than lot-to-lot variations from a single supplier.

Although fetal bovine serum is a natural product and should be relatively constant, different lots seem to vary in levels of certain hormones present (5) and in ability to support cell attachment and growth, as reported herein. The differences between sera could arise from different handling procedures after collection. Such a possibility is consistent with the report (1) that fetal bovine serum prepared under carefully controlled conditions was significantly better in that serum; if kept in the same serum for determining plating efficiency, a higher number of colonies might result.

Table 3. Absolute plating efficiencies of sera using cells from primary cultures grown with different sera

| Secondary sera | F-4 | F-5 | E-2 | E-1 |
|----------------|-----|-----|-----|-----|
| F              | 12.9| 12.5| 11.6| 13.1|
| E              | 12.4| 11.4| 11.6| 10.6|
| E              | 13.2| 11.5| 11.4| 12.1|
| F              | 12.8| 14.6| 13.9| 14.0|
| E              | 11.3| 8.2 | 11.9| 9.8 |
| Avg            | 12.5| 11.6| 12.1| 11.9|

* Only two of five cloning plates could be counted.
* ND, This group of five plates was lost due to contamination.

Table 4. Effect of initial outgrowth serum on absolute plating efficiencies of hamster embryo cells

| Secondary serum | Outgrowth serum |
|----------------|----------------|
|                | High (control) | Medium (C2) | Low (B5) |
| High: control  | E3             | 103          | 80       | 122     |
| Medium: C2     | G4             | 51           | 41       | 76      |
| Low: C1        | B5             | 19           | 11       | 22      |
|                | 11             | 5           | 6        |
different from standard commercial lots, even by the less-sensitive method of plating cells at higher densities.

In performing the experiments reported here, it was not possible to judge for the majority of "average" lots whether the decreased absolute plating efficiency was due to toxic materials in the serum or to decreased nutrients. The sera with very low plating efficiency, however, appeared to be toxic to the cells. If so, this toxicity could arise, for example, from growth of bacteria with release of toxic products, prior to the sterilization of the serum by filtration.

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