

Application Forum

Decreasing variability in your cell culture

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Culturing mammalian cells has not significantly changed in almost 50 years. Typically, a synthetic basal medium is chosen to meet the environmental and nutritional requirements of a given cell line. Components, such as amino acids, vitamins, inorganic salts, and a carbon source such as glucose are commonly found in the classical basal media formulation. These basal formulations normally will not support cell growth alone, but must be further supplemented with animal serum, usually fetal bovine serum (FBS) at a concentration of 5–20%. Recent advances in serum-free and chemically defined media formulations have provided cell culturists with options. When considering FDA regulations and potential risks to human health when manufacturing biologics or considering cell therapies, eliminating serum is of paramount concern. For a large majority of researchers however, using classical media with serum builds on previous generations of research and makes cell culture easier to perform.

COMPONENTS OF SERA

Although serum is vitally important to the growth of cells cultured in a classical basal medium, it has never been fully characterized. There are over 1,000 different components found in serum¹, including proteins, electrolytes, lipids, carbohydrates, hormones, enzymes, and other miscellaneous undefined constituents. Serum also supplies growth factors, nutrients for proliferation and differentiation, factors for binding to and inactivating toxic compounds such as proteases and free radicals, and attachment factors.

In addition, serum also regulates cell membrane permeability and serves as a carrier for lipids, enzymes, micronutrients, and trace elements into the cell. Finally, albumin, fetuin, and other bulk proteins found in serum minimize nonspecific absorption to culture-ware and bioreactor surfaces, as well as influence the physical properties of the culture system, such as pH (sera are a source of natural buffers), shear stress, viscosity, osmolality, and gas delivery rates².

CHOOSING A SERUM

The best serum is one that works for your cells in your application, is readily available from suppliers, and fits your budget. Different geographic sources provide advantages around certain viral risks, however manufacturers have improved the quality and breadth of their testing such that you can be assured that your cultures will not be negatively affected by viruses. If you're working as part of a consortium or in collaboration with researchers in different geographic areas, source becomes increasingly important

as government regulations restrict importation/exportation of various geographic sources. It will be very important to your combined work to ensure that you've standardized on a single lot/source of serum as testing between different sources varies.

As technologies continually improve and provide us with new ways to study and understand cells, so do our requirements from the products that support and/or affect growth to reduce variability and minimize signal to noise ratios.

WHY IS SERA VARIABLE?

All sera represent an undefined mixture in which composition varies from one lot to the next. Sensitive cell culture methods designed to collect quantitative experimental data may be seriously affected by the variability of the constituents in sera. For example, hormones are known to exert profound effects at concentrations in the pico- and nanogram ranges, effects that may be pervasive without respect to cell type³; yet, serum hormone concentrations vary from lot to lot. In Honn, et al. (1975), four metabolites were evaluated in terms of their variability and possible toxicity to cell cultures¹.

Analysis of uric acid, urea, total bilirubin, and creatine concentrations indicated a two to six-fold variation in sera samples tested. Cholesterol has been reported as important as an attachment factor for increased plating efficiency⁴; however, cholesterol levels varied in different lots of FBS tested (29–165 mg/dl), suggesting that reliance upon different lots of sera supplements for consistent plating efficiency is tenuous. The concentration of glutamate in serum may also be toxic to cultured neuronal cells.

HOW SERA VARIABILITY AFFECTS RESEARCH?

Stimulation or inhibition of growth

Factors can be present in any given lot that either stimulate or inhibit the desired culture response of specific cell types. Inhibition of growth and/or cellular function can be related to the level of specific serum constituents, such as amine oxidases⁵. The life span of diploid cells has also been shown to vary with different serum batches⁶.

Unwanted induction of differentiation

Factors in sera may also induce differentiation of progenitor cells, which may be counterproductive to the scientist's intent. Even when cultured under serum-free conditions, quenching the action of trypsin with a serum-supplemented medium may cause differentiation, because of high concentrations of calcium typically found in FBS.

MINIMIZING VARIATIONS

Prequalifying and reserving sera

One way that scientists can overcome the problem of lot-to-lot variability is by prequalifying and reserving sera for their specific applications. The traditional methodology most often chosen is a proliferation or differentiation assay on one or two cell lines. Often times, these cell lines are not representative of the intended application although a qualification based on proliferation puts many researchers at ease.

Reserving a large lot of material is costly and locks customers in at a set price. Should manufacturing costs and market prices decrease, researchers who have established large sera reserves can find themselves in the unfortunate position of 'owning' inventory that is considerable more expensive than product they could purchase in the open market.

Additionally, prequalification of sera:

- Takes considerable investment (time and money) from a laboratory technician or research associate
- Decreases focus on scientific discovery

Lot matching

Sera lot-matching programs provided by manufacturers allow for lots with similar performance and biochemical specifications to be purchased as replacements for sera lots that have worked well for specific applications in the past. Lot testing is a relatively new technique to provide researchers with pared-down samples/candidates that have a higher probability of working in their application. Lot-

matching programs help scientists target potentially suitable sera lots for their own applications; however, many scientists will also prequalify samples to further ensure that the proper lot of sera has been chosen.

Over the past 10 years lot manufacturers provided lot matching to scientists by first searching available inventory to ensure availability of the chosen quantity. A manual lot match was then conducted by serum technical specialists examining 2 or 3 Certificate of Analysis parameters (usually endotoxin, hemoglobin, and a performance assay). Recommended samples were sent to customers for qualification and researchers would then contact manufacturers and request shipment of the entire purchase or establish reserves with regular shipments.

Multi-parametric analysis (MPA) lot matching

Recent advances in bioinformatics have led manufacturers to offer additional options for lot matching. Since wide arrays of tests are conducted in order to release a lot of serum from production, some manufacturers can now conduct multi-parametric analysis lot matching for scientists.

Growth, plating, and cloning assays provide valuable information on growth kinetics. Osmolality, pH, hormone, antibody, hemoglobin, and endotoxin measurements provide definitive measurements that directly influence culture performance. Having the ability to compare all of these parameters with weighted averages yields a 'virtual fingerprint' of a specific lot of serum. Using this as a baseline for comparison, manufacturers can now provide improved samples and/or guarantees on performance for researchers' applications, negating the need to test and establish large reserves. Recently, GIBCO® introduced iMATCH™ — an MPA lot matching tool which provides FBS specialists with the ability to analyze up to 50 different factors and conduct an MPA lot match in seconds.

"iMATCH™ is revolutionizing the way scientists make serum choices," comments Aaron Stein, Business Area Manager at GIBCO®/Invitrogen. "Scientists and manufacturers have never had such a powerful tool focused on enabling research and minimizing variability. The result is that researchers are now able to purchase serum with increased confidence, that it will work in their application. Scientists can now spend more time on discovery and less on nonvalue added tasks like testing serum. iMATCH™ is GIBCO®'s assurance to customers that they are getting the best material available for their cells, in their application." In addition to the full complement of tests regularly conducted on sera, beginning in February 2007, GIBCO® will be going one step further to characterize its sera by conducting proliferation screening assays on every lot of US and USDA serum for the following cell lines.

- CHO-K1 (hamster kidney)
- 293F (Human embryonic kidney)
- Jurkat (human T-cell leukemia)
- THP-1 (Human monocyte)
- ME-180 (human cervical carcinoma)

Stein adds, “by conducting these 5 additional research assays, we have taken a major step towards characterizing serum. We’re not trying to reverse-engineer serum—that would be a fruitless effort. Instead, we’re watching and measuring cells reaction/performance to an individual lot of serum and comparing it to previous results. Our iMATCH™ informatics tool creates a virtual fingerprint of every lot, and with researcher input, enables us to provide our customers with a lot of serum that will work in their application.”

SUMMARY

Researchers have traditionally relied on the benefits of animal sera, primarily FBS, to support their cell cultures in spite of the known disadvantages associated with the use of sera. The issues surrounding sera have led scientists to explore such options as prequalifying and reserving sera, lot matching, and now MPA lot matching, all which allow more effective management of sera usage and requirements. With the goal of having a defined media formulation for every cell type and every research application being down the road, manufacturers like GIBCO® are leading the way in our understanding of serum.

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