Benchtop cell-health monitoring

A baseline for cell-based screening assays

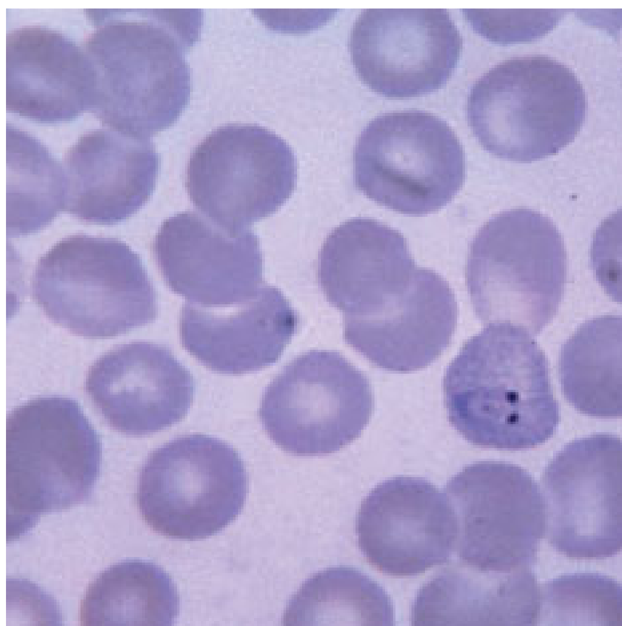
BY LAWRENCE LEM AND KEITH OLSON

The pharmaceutical industry faces no shortage of novel targets and promising drug compounds. Combinatorial chemistry, genomics, and proteomics are uncovering unprecedented numbers of possibilities that require screening. Although biochemical assays provide useful information on both biological targets and compound activity, whole-cell assays remain crucial in drug discovery screening. As intact living systems, whole cells can reveal subtle but important effects from compounds not seen or predicted in biochemical assays. However, this same responsiveness of whole cells can also prove to be an unpredictable factor in cellular drug screening assays if the health of the cells changes during the course of the assay.

Live-cell cultures are dynamic, and the proportion of viable, dead, and apoptotic cells continuously fluctuates as a culture grows. Some drug screening procedures require that compound incubations be carried out for several days, and during this period, the percentage of viable cells may fall while the percentage of apoptotic cells may rise, for many reasons. As apoptotic cells progress through programmed cell death, they display altered metabolisms that may respond differently to treatment with compounds. Thus, a substantial increase in the number of apoptotic cells may skew the results of screening experiments.

In cytotoxicity screening, for example, a rising percentage of apoptotic cells may contribute to artificially high positive results, which may be misinterpreted as cytotoxicity of the test compound. In the worst possible situation, apoptotic cells may reach levels high enough to invalidate the screening assay. One way to control for this fluctuation is to monitor the health of untreated cell cultures throughout the course of screening experiments to establish a true baseline of the viable cell population.

Although they do not necessarily indicate a cell population in failing health, the data in Figure 1 illustrate the importance of regular monitoring of cell cultures prior to their use in screening assays. In this instance, the cells in question were engineered to express an epitope-tagged protein that could be monitored with a simple antibody test, and the data collected demonstrated that expression had fallen to unacceptable levels on certain days during the month. Because of this monitoring, it was possible to avoid using these cells, saving valuable time and money.



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Monitoring methods

The monitoring of cell culture health, including apoptosis and viability assessments, is not a commonplace procedure during drug screening. For cell counting and viability assessments to be useful for quality control of cell screening assays, the method must be simple, fast, and accurate in its ability to count cells. In addition, the technique will have added value if it can simultaneously detect and count both apoptotic cells and dead cells, revealing early signs of deterioration in cell health that may be missed if apoptosis is ignored. All of these data must be obtained without requiring the maintenance of large cell cultures in addition to the screening plates.

Although several methods exist for assessing cell viability, none of them are expedient for quality control of screening assays. Trypan blue exclusion assays have traditionally been the favored method for counting viable cells. Because live cells exclude the dye, they are easily distinguished from blue-stained, dead cells. However, the assay requires categorizing stained and unstained cells, usually by visual determination with a microscope, so problems arise when cells show intermediate staining levels. Varied interpretation of these intermediately stained cells leads to significant inter-operator variation in trypan blue cell counts (*1*). In fact, trypan blue counts often overestimate viable cell populations because apoptotic cells sometimes exclude dye even as they progress through apoptosis (*2*). Automated versions of the trypan blue assay provide more consistency but often require culture samples in the milliliter range, making it impractical for repeated sampling over the time course of a screening experiment.

Cell counting and monitoring can also be done on traditional flow cytometers. These viability assays are based on DNA stains such as propidium iodide. However, the assays provide only percentages of live and dead cells. Acquiring absolute cell counts on a cytometer requires using expensive reference beads as standards for counting. The sensitivity of flow cytometers does allow intermediately stained cells to be quantified as apoptotic cells. However, the typical sample size is about 1 mL, far too large for convenient

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counting of large numbers of samples and repeated sampling over time. Moreover, the expertise required to run flow cytometer experiments makes them inaccessible to most screening departments.

Fast and efficient

Several new assays from Guava Technologies, combined in a personal cell analysis system, have the speed and convenience of mix-and-read protocols and the ability to assess cellular health. Using standard 96-well microplates, cell health can be evaluated through information on apoptotic populations, viable cell counts and proliferation data, and even cell cycle progression data.

The Guava ViaCount Assay distinguishes viable, nonviable, and apoptotic cells on the basis of differences in the permeability of two fluorescent dyes. One dye stains DNA in all nucleated live and dead cells, whereas the viability dye stains only dead cells with porous membranes. Cellular debris and cells without a nucleus are not stained, so they are not erroneously counted. The two dyes fluoresce at different wavelengths; live cells appear red and dead cells stain both red and orange. This combination of dyes provides counts of live and dead cells.

In most cell lines, apoptotic populations take up intermediate amounts of the dead-cell dye, as shown by correlated staining with annexin V, another indicator of apoptosis (*3*). Small changes in cell populations can also be identified with the ViaCount assay. Comparisons of precision with trypan blue/hemacytometer counts indicate that Guava ViaCount results have at least a threefold lower coefficient of variation (CV) than manual hemacytometer counts (*4*). The increased precision of the ViaCount assay comes without significant time expenses. The assay requires only the mixture of the cell sample and a single reagent, followed by 5–15 min of incubation. With this simplicity, the assay provides all the speed, ease, and information needed for an effective monitoring assay.

A second fast and simple assay that provides extremely reliable apoptosis information is the Guava Nexin assay. During the early stages of apoptosis, phosphatidylserine that is normally located on the inner face of the plasma membrane is translocated to the external face. This event can be easily detected by surface labeling with fluorochrome-conjugated annexin V, which readily binds to phosphatidylserine. Requiring only the mixing of a single reagent and a short staining period, the Guava assay is simple and can easily be performed as a monitoring assay.

Combining Nexin and ViaCount allows scientists to easily and efficiently obtain comprehensive views of cell health at various time points during a screening assay. With the combination of assays, several measures of cell health can be assessed. ViaCount assays provide accurate counts of both live and dead cells, so over a period they provide information on proliferation rate in addition to cell viability. Guava Nexin reliably indicates the apoptosis levels in a culture; in many situations, intermediately stained cells in ViaCount assays also indicate apoptosis in culture. If information on cell cycle distribution is needed, the Guava Cell Cycle assay can be performed in addition to these assays. The Guava Cell Cycle assay uses measurements of cellular DNA content to determine the proportion of cells in each phase of the cell cycle. These measurements are based on fluorescent detection of DNA-binding dues used to stain the cells. Because these three assays require only microvolumes of cell sample, controls can be conveniently cultured in a single 96-well plate, even if controls are required in quadruple replicates.

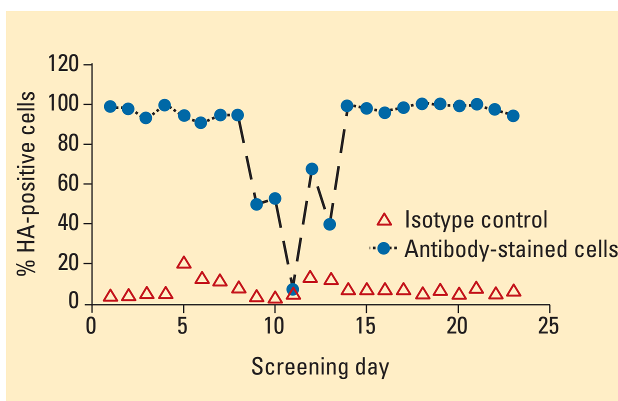

Figure 1. Avoiding pitfalls. By monitoring cell cultures with a simple antibody test, researchers could avoid using engineered cells—expressing an HA-tagged cell surface protein—on days when expression dropped to unacceptably low levels. The cells were stained with an anti-HA tag antibody, labeled with a secondary antibody, and analyzed versus control samples on the Guava PCA. (Data courtesy of R. Garippa, Hoffmann-La Roche Ltd.)

Accounting for apoptosis

Knowing the background level of apoptotic cells throughout the drug discovery and screening process becomes increasingly important as recent studies have found that natural and synthetic compounds affect apoptosis. For example, resveratrol, an anticancer agent in grapes, is cytotoxic to HL-60 promyelocytic leukemia cells and induces apoptosis (*5*). The newly discovered process of interference RNA promotes apoptosis in hepatocellular carcinoma cells, thereby blocking cell proliferation (*6*). Moreover, standard pharmacology measurements, such as the drug concentration required to kill 50% of tumor cells (EC_{50}), do not take into account the propensity of cells to undergo apoptosis.

The assays discussed here enable researchers to establish baseline viabilities against which to compare their screening results, helping them avoid misinterpreting cytotoxicity or other data that are actually a result of unexpected or "artifactual" apoptosis. This is particularly important when the proportion of apoptotic cells changes during the time course of a screening. The assays' ease, speed, and convenience allow them to be easily adopted as monitoring methods in any screening process without significantly altering established protocols.

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