ABSTRACT

The tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), is reduced by live but not dead cells, and this reaction is used as the end point in a rapid drug-screening assay. It can also be used for accurate determinations of drug sensitivity but only if a quantitative relationship is established between cell number and MTT-formazan production. We have shown that reduction of MTT to MTT-formazan by cells is dependent on the amount of MTT in the incubation medium. The concentration required to give maximal MTT-formazan production differs widely between cell lines. The absorption spectrum of MTT-formazan varies with cell number and with pH. At a low cell density or a high pH, the absorption maximum is at a wavelength of 560 to 570 nm. However, at a high cell density or a low pH, there are two absorption maxima; one at 510 nm and a second at about 570 nm. Measurements of absorbance at 570 nm underestimate MTT-formazan production and, hence, cell number at high cell densities. This error can result in a 10-fold underestimation of chemosensitivity. Addition of a buffer at pH 10.5 to the solubilized MTT-formazan product can overcome the effects of both cell density and culture medium on the absorption spectrum. Provided that sufficient MTT is used and the pH of the MTT-formazan product is controlled, dye reduction can be used to estimate cell numbers in a simple chemosensitivity assay the results of which agree well with a commonly used clonogenic assay.

INTRODUCTION

Although there are a number of chemosensitivity assays suitable for use in vitro, few are applicable to all cell types, and there is no universally accepted assay. Interest in a tetrazolium dye-based assay has been stimulated following the adoption of such an assay by the National Cancer Institute for use in its drug-screening program (1, 2). The assay relies on the ability of live but not dead cells to reduce a water-soluble yellow dye, MTT, to a water-insoluble purple formazan product. Since the substrate and product absorb at very different wavelengths, no washing steps are required after removal of the aqueous medium prior to solubilization of the MTT-formazan product. This is a clear advantage over those assays which use isotope incorporation as an end point, particularly when dealing with non-adherent cell lines. As designed for drug screening, the assay requires minimal manipulation and is easily automated (1). It is thus ideally suited to a program that involves a wide range of cell types and thousands of potential anticancer agents. However, when the assay is used to determine drug sensitivity, a number of shortcomings are apparent. MTT-formazan production is used as an estimate of surviving cell number following exposure to a cytotoxic drug. Many attempts to use the assay have failed to demonstrate a linear relationship between MTT-formazan production and cell number at high cell densities (1, 3, 4). This is particularly important, since the highest cell numbers occur in the control, untreated wells, and these are used to determine the parameters of sensitivity to the cytotoxic drug.

We have now established conditions under which MTT reduction can be used quantitatively to determine surviving cell numbers in a chemosensitivity assay that can be applied to both adherent and nonadherent cell lines. This assay has been compared with an alternative and widely used drug sensitivity assay.

MATERIALS AND METHODS

Cell Lines

Four non-small cell lung cancer cell lines were used: A549, CALU, and SK-MES obtained from the American Type Tissue Collection; and L-DAN, a squamous lung cancer cell line established in our own laboratory. The doubling times of the lines were about 24 h. The other two adherent cell lines were MCF-7, a breast cancer line obtained from Dr. K. Cowan, National Cancer Institute, and G-UVM, a glioma cell line established in our own department. The doubling times were about 24 and 60 h, respectively. Two nonadherent cell lines were used. These were two small cell lung cancer lines: GLC6, obtained from Dr. E. deVries, Department of Clinical Oncology, University of Groningen, The Netherlands; and NCI-H187, obtained from Dr. D. Carney (5). The doubling times were about 24 and 48 h, respectively.

Cells were maintained in a mixture of Ham’s F10 and DMEM (50:50; Gibco, Paisley, Scotland) supplemented with glutamine (2 mM) and fetal calf serum (10%, v/v) except for MCF-7, GLC6, and NCIH69. These lines were maintained in RPMI 1640 (Northumbria Biologicals, Cramlington, Northumberland, England) supplemented with glutamine (2 mM) and fetal calf serum (10%, v/v).

Determination of Absorption Spectra

MTT and MTT-formazan (Sigma Chemical Company, Poole, Dorset, England) were dissolved in DMSO, and the absorbance was scanned between wavelengths of 350 and 700 nm. MTT-formazan produced by cells was obtained by incubation of cells in the presence of MTT for 4 h. MTT was removed and the formazan crystals dissolved in DMSO.

Determination of the Optimal MTT Concentration

Cells were plated out in 200 μl of medium at a concentration of either 105 cells per flat-bottomed well or 5 x 104 cells per round-bottomed well (GLC, and NCI-H187) in 96-well microtiter plates (Linbro; Flow Laboratories, Irvine, Scotland). The first and last row of 8 wells contained medium only. Plates were incubated for 24 h at 37°C in an atmosphere of 2% CO2 in air. MTT (0 to 5 mg/ml) was dissolved in PBS, and 50 μl were added to each well (8 wells per concentration). Plates were wrapped in aluminum foil and incubated for a further 4 h. Medium and MTT were then removed from the wells, and the formazan crystals were dissolved in 200 μl of DMSO. In some experiments, 25 μl of Sorenson’s glycine buffer (0.1 M glycine plus 0.1 M NaCl equilibrated to pH 10.5 with 0.1 N NaOH) were added at this stage. Plates containing nonadherent cells were centrifuged at 200 x g for 5 min prior to removal of the medium. The absorbance was recorded in an enzyme-linked immunosorbent assay plate reader (Model 2550 enzyme immunoassay plate reader; Bio-Rad Laboratories, Ltd., Watford, Eng-
was incubated for 4 h. The medium was re-added (8 wells per concentration), and the plate dissolved in DMSO. Bars, SE.

MTT (SO>ilof a solution of 0 to 5 mg/ml) was dissolved in DMSO. Also shown is the spectrum of MTT-formazan produced by incubation of L-DAN cells (4000 per well) with MTT (5 mg/ml) for 4 h.

Fig. 1. Absorption spectra of MTT and commercially prepared MTT-formazan dissolved in DMSO. Also shown is the spectrum of MTT-formazan produced by incubation of L-DAN cells (4000 per well) with MTT (5 mg/ml) for 4 h.

Relationship between MTT-Formazan Production and Cell Number

Cells were diluted to a concentration of 2.5 x 10^5 cells per ml. Various volumes of this cell suspension (20 to 200 µl) were plated out in duplicate 96-well microtiter plates (8 wells per volume). Plates were incubated for at least 24 h at 37°C in an atmosphere of 2% CO2 in air. To one plate, 50 µl of MTT in PBS (concentration depending on cell line) were added, and the plate was processed as described above. In this case, the first row of 8 wells, which contained medium and PBS (50 µl) only were used to blank the plate reader.

RESULTS

Spectral Analysis. The absorption spectra of MTT and MTT-formazan are shown in Fig. 1. Also shown is the absorption spectrum for MTT-formazan produced by incubation of cells in the presence of MTT. For MTT, there is a single absorption maximum at a wavelength of 410 nm. MTT-formazan exhibits two absorption maxima at wavelengths of about 510 and 570 nm (see also Fig. 5). However, the MTT-formazan produced by incubation of cells with MTT demonstrates a single absorption maximum at a wavelength of about 560 nm.

Effect of MTT Concentration on MTT-Formazan Production. Fig. 2 shows the amount of MTT-formazan produced when the cell line L-DAN is incubated with various concentrations of MTT. MTT-formazan production increases with increasing MTT concentration up to about 4.5 mg/ml. This effect was observed regardless of whether cells were grown in F10/DMEM or in RPMI 1640 medium. A similar relationship is also apparent for the small cell lung cancer cell line, GLC4. However, for this cell line, the formazan production achieves a plateau level at an MTT concentration of only 1.2 mg/ml. Similarly, for the cell line, NCI-H187, a plateau is achieved at an MTT concentration of only 1.8 mg/ml, whereas for the cell line,
TETRAZOLIUM-BASED CHEMOSensitivity ASSAY

Fig. 3. Relationship between MTT-formazan production and cell number for the cell lines L-DAN and GLC4. Cells were plated out at a range of concentrations and incubated for 24 h. Cell number was then estimated either by counting following trypsin treatment if required or by incubation with MTT (L-DAN, 5 mg/ml; GLC4, 2 mg/ml) for 4 h.

Fig. 4. Absorption spectrum of MTT-formazan produced by incubation of A549 cells with MTT (5 mg/ml) for 4 h at low (4 × 10^3 cells per well) and high (10^4 cells per well) cell densities. MTT-formazan crystals were dissolved in DMSO.

MCF-7, the concentration is about 4.25 mg/ml (results not shown).

Relationship between MTT-Formazan Production and Cell Number. The relationship between MTT-formazan production and cell number for the cell lines L-DAN and GLC4, is shown in Fig. 3. There is a linear relationship up to a cell number of about 2 × 10^4 cells per well. However, it should be noted that neither regression line extrapolates back through the origin.

Effect of Cell Number on the Absorption Spectrum of MTT-Formazan. Fig. 4 shows the absorption spectrum of MTT-formazan produced following incubation of cells in the presence of MTT. For low cell numbers (4 × 10^3 cells per well), the spectrum shows a single absorption maximum at a wavelength of about 570 nm. In contrast, if the cell number is increased to 10^4 cells per well, the peak is much broader and the absorption maximum is at a wavelength of about 510 nm. There is also a shoulder in this peak at a wavelength of about 580 nm.

Effect of pH on MTT-Formazan Absorbance. Fig. 5 shows the absorption spectrum of a solution of MTT-formazan in DMSO (200 µl) in the presence of glycine buffer (25 µl) equilibrated at a range of pH values. For pH 7.5, a phosphate buffer was used.

Effect of Buffer Addition on the Relationship between MTT-Formazan Production and Cell Number. Fig. 6 shows the relationship between MTT-formazan production and cell number for the cell line A549 both before (O) and after (•) addition of buffer to the wells (25 µl, 0.1 M, pH 10.5). The absorbance of the MTT-formazan is increased for all cell numbers, but the increase is most marked at high cell numbers. Furthermore, following addition of buffer, the regression line extrapolates back through the origin. Addition of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (10 mM, pH 7.4) during the incubation with MTT did not alter the slope of the line but reduced the scatter of points about the line (results not shown). Although results are shown for A549, this effect was observed with all cell lines studied.

Effect of Culture Medium on MTT-Formazan Absorption. Table 1 shows the effect of different volumes of culture medium (F10/DMEM containing 10% fetal calf serum) on the absorbance of a standard solution of MTT-formazan. Addition of culture medium results in an increase in the absorbance of the MTT-formazan, but the increase is greater for small additions raised, the spectrum shifts to the right and a single absorption maximum, at about 560 nm, is observed above pH 8.5. The height of the peak increases with increasing pH and reaches a maximum at pH 10.5.

Fig. 5. Absorption spectrum of a solution of commercially prepared MTT-formazan in DMSO or in DMSO (200 µl) plus glycine buffer (25 µl) equilibrated at a range of pH values. For pH 7.5, a phosphate buffer was used.

Fig. 6. Effect of buffer addition on the relationship between MTT-formazan production and cell number. Cells (A549) were plated out at a range of concentrations and allowed to adhere for 24 h. Cells were then either trypsinized and counted or incubated with MTT (5 mg/ml) for 4 h. Medium was removed, and MTT-formazan crystals were dissolved in DMSO, and the absorbance was recorded at 570 nm (O). Buffer (25 µl, 0.1 M glycine, pH 10.5) was added to each well, and the absorbance was again recorded at 570 nm (•).
concentrations of either doxorubicin or vincristine for 24 h. MTT-
duction per well 3 days after exposure of cells to various
addition of up to 50 μl of medium results in only a very small
increase in absorbance in the presence of 5 μl of culture medium,
than for large additions. Table 1 also shows the absorbance
values obtained after addition of buffer (25 μl per well, 0.1 m, pH
10.5) to the same plate. Although there is still a slight
increase in absorbance in the presence of 5 μl of culture medium,
addition of up to 50 μl of medium results in only a very small
reduction in MTT-formazan absorption. It should be noted that
addition of buffer to the MTT-formazan alone resulted in a
marked increase in the absorbance.

Effect of Buffer Addition on the Apparent Chemosensitivity to
Doxorubicin and Vincristine. Fig. 7 shows MTT-formazan pro-
duction per well 3 days after exposure of cells to various
concentrations of either doxorubicin or vincristine for 24 h.
when the MTT-formazan crystals were dissolved in DMSO
and the absorbance noted at a wavelength of 570 nm, the ID50
for doxorubicin for the cell line L-DAN was 1.8 × 10^-7. A
similar effect was observed for the other cell lines (results not
shown) and when vincristine was used (Fig. 7).

**DISCUSSION**

The end point of a chemosensitivity assay is usually an
estimate, either direct or indirect, of surviving cell numbers.
Use of a tetrazolium dye as the end point relies on the ability
of cells to reduce the dye in a quantitative manner. We have
shown that, when cells are incubated with MTT, the amount
of MTT-formazan produced depends upon the concentration
of MTT in the incubation medium. MTT-formazan production
increases with increasing MTT until a concentration is reached
at which MTT-formazan production is maximal. This optimum
concentration differs widely among cell lines. For example, for
the two small cell lines, NCI-H187 and GLC4, and of the adherent cell lines to doxorubicin
as determined by the microtitration assay. The overall error of
the estimates is small (coefficient of variation, 18%) and is
similar for both adherent and nonadherent cell lines. Also
shown in Table 3 are the sensitivities of the adherent cell lines
to both doxorubicin and vincristine determined both by the
microtitration assay and by a standard clonogenic assay. The
standard errors for the two assays are similar, and there is close
agreement between the two assays for all cell lines and for both
drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cell line</th>
<th>Microtitration</th>
<th>Clonogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>NCI-H187</td>
<td>36 ± 2 (6)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLC4</td>
<td>12 ± 3 (6)</td>
<td></td>
</tr>
<tr>
<td>L-DAN</td>
<td>23 ± 4 (7)</td>
<td>24 ± 7 (6)</td>
<td></td>
</tr>
<tr>
<td>A549</td>
<td>23 ± 4 (7)</td>
<td>38 ± 7 (6)</td>
<td></td>
</tr>
<tr>
<td>WIL</td>
<td>57 ± 6 (7)</td>
<td>34 ± 8 (4)</td>
<td></td>
</tr>
<tr>
<td>SK-MES</td>
<td>33 ± 2 (10)</td>
<td>21 ± 7 (4)</td>
<td></td>
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<tr>
<td>CALU</td>
<td>11 ± 1 (6)</td>
<td>5 ± 3 (3)</td>
<td></td>
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<tr>
<td>G-LUVW</td>
<td>19 ± 6 (4)</td>
<td>16 ± 9 (4)</td>
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<tr>
<td>MCF7</td>
<td>28 ± 1 (3)</td>
<td>30 ± 4 (3)</td>
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<tr>
<td>Vincristine</td>
<td>L-DAN</td>
<td>12 ± 1 (6)</td>
<td>12 ± 2 (6)</td>
</tr>
<tr>
<td>A549</td>
<td>44 ± 10 (5)</td>
<td>22 ± 4 (5)</td>
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</tr>
<tr>
<td>WIL</td>
<td>41 ± 4 (7)</td>
<td>20 ± 8 (4)</td>
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</table>

* Mean ± SE.
Numbers in parentheses, number of estimations.
of about 2 mg/ml (0.4 mg/ml final) gives maximum MTT-formazan production, whereas for the non-small cell lines and for MCF-7, a concentration of about 5 mg/ml (1 mg/ml final) is required (Fig. 2). This difference cannot be explained in terms of the medium used, since it is seen between the small cell lines and MCF-7, all of which were grown in RPMI 1640 medium. Furthermore, the concentration of MTT required to give maximum MTT-formazan production by L-DAN was independent of the medium used. The screening assay (1) uses a final concentration of 0.4 mg/ml for all cell lines, and this is the amount used in the original assay (3), although it has been observed that, for a T-lymphoma cell line, an MTT concentration of 1 mg/ml gives optimal MTT-formazan production (6).

We have found that, for most cell lines, a final concentration of 1 mg/ml is more than sufficient, but we cannot recommend this optimal for the assay, since we have observed that for some cell lines an excess of MTT can result in a reduction in MTT-formazan production.

Previous attempts to use tetrazolium dye reduction as an estimate of cell number have failed to demonstrate a linear relationship between the amount of dye reduced and cell number, particularly at high cell numbers and absorbance values of greater than 0.8 (1, 3, 4). Our observations demonstrate that, if the optimum MTT concentration is determined, a linear relationship exists between cell number and MTT-formazan production up to a cell number of about 2 x 10⁶ cells per well (Fig. 3). However, when the regression line is extrapolated back, it does not pass through the origin such that MTT-formazan is apparently produced in the absence of any cells. Since both medium and MTT are used to establish the background absorbance when reading the plate, this observation might indicate that the true relationship is not linear. It should be noted that points can often be joined by a straight line when a curve is clearly a better fit (for example, see Fig. 1; Ref. 4).

It was noted that commercially prepared MTT-formazan is not the same color when dissolved in DMSO as that produced by incubation of cells in the presence of MTT. This observation was confirmed by spectral analysis of the two MTT-formazans. Commercially prepared MTT-formazan exhibits a major absorption maximum at a wavelength of 510 nm and a shoulder in the peak which may represent a second absorption maximum at around 560 to 580 nm. In contrast, that produced by cells (4000 per well) exhibits a single absorption maximum at 560 to 570 nm (Fig. 1). This difference has also been reported elsewhere (2) but was attributed to the presence or absence of serum when MTT-formazan is dissolved in DMSO. This cannot be the only explanation, since we have shown that, if the cell number is increased to 10⁶ cells per well, contaminating serum is still present, yet the absorption spectrum of the MTT-formazan product resembles that of the commercially prepared MTT-formazan (Fig. 4). A similar effect is seen when medium is added to a solution of MTT-formazan in DMSO. Addition of a small volume of medium (5 to 10 µl) results in a much greater increase in absorbance at 570 nm than does addition of a larger volume (20 to 50 µl). Furthermore, our observations indicate that the absorption spectrum of MTT-formazan is pH dependent. At an acid pH (3.5), the spectrum resembles that of the commercially prepared MTT-formazan in DMSO alone, and the absorbance measured at 570 nm is low. However, if the pH of the solubilized MTT-formazan is increased, the spectrum approaches that of the MTT-formazan produced by cells (4000 cells per well; Fig. 4 cf. Fig. 5), and the absorbance at 570 nm is markedly increased. At a pH of 10.5, the absorption spectrum exhibits a single peak at 570 nm, and the absorbance at 570 nm reaches a plateau value which is not affected by any further increase in pH (Fig. 5).

We cannot explain the changes in the absorption spectrum of MTT-formazan. When MTT is reduced, the tetrazolium ring is opened and the quaternary amine is converted to a tertiary amine. A second tertiary amine which formed one of the bonds to the quaternary amine becomes bonded to a hydrogen atom. It could be hypothesized that displacement of this hydrogen at high pH results in changes in the absorption spectrum of the molecule. Whatever the explanation, the spectral shifts produce a real problem when dye reduction is used to estimate cell numbers. At low cell numbers, the absorption maximum is at about 560 to 570 nm, while at high cell numbers there are two peaks: the larger at 510 nm and a smaller peak at 570 nm. Measurement of absorbance at a single wavelength is thus inappropriate. In an attempt to overcome this problem, we raised the pH of the MTT-formazan product to 10.5. At this pH, a single peak is observed regardless of cell number or the presence of culture medium. Addition of a small quantity of buffer at pH 10.5 to the solubilized MTT-formazan product resulted in a significant increase in the absorbance reading for wells with a high cell density (50% increase for 3 x 10⁴ cells per well; Fig. 6). Furthermore, it resulted in a clear linear relationship between MTT-formazan production and cell number up to cell numbers of 5 x 10⁴ per well and absorbance readings of 1.5 (Fig. 6). This range was well within the limits required for a chemosensitivity assay even for cell lines with a rapid doubling time. It is of interest that a strong base has been used to enhance the sensitivity of the nitroblue tetrazolium test, but in this instance, it was claimed to increase the solubility of the formazan product in DMSO (7).

The importance of these observations becomes apparent when MTT reduction is used to estimate cell numbers in a chemosensitivity assay. If the MTT-formazan crystals are dissolved in DMSO alone, the control cell number is markedly underestimated and the apparent ID₅₀ is about 10-fold higher than is obtained by a standard clonogenic assay (Fig. 7 cf. Table 3). Addition of buffer (pH 10.5) to these same wells resulted in a significant increase in the estimated cell number in the control wells and in wells exposed to low concentrations of doxorubicin such that the apparent ID₅₀ agrees well with that obtained by the clonogenic assay (Fig. 7 cf. Table 3).

Previously, it has been observed that MTT-formazan absorption is greatly increased in the presence of 10 µl or less of culture medium but only slightly increased in the presence of 10 to 40 µl of medium (8). We also observed this effect (Table 1). However, addition of the buffer at pH 10.5 causes a significant increase in the absorbance of MTT-formazan and overcomes most of the variability in absorbance reading caused by the presence of culture medium (Table 1). This is an important observation, since for the nonadherent cell lines, it is not possible to remove all of the medium from the wells prior to the addition of DMSO. In the screening assay, it was noted that the variability between wells was greater for nonadherent cell lines, and it was suggested that the residual medium left in the wells may be responsible (1). We do not observe any increase in variability with nonadherent cell lines, and this could be explained by the inclusion of the buffer at the final stage of the assay. Although it was suggested that this problem would be overcome by the introduction of an alternative tetrazolium dye which is reduced to a water-soluble formazan product (XTT), there are major problems encountered in the use of this dye (9). Control of the pH during incubation of cells with MTT, through incorporation of 4-(2-hydroxyethyl)-1-piperazineethanesul-
fonic acid buffer into culture medium used for this step, also decreased the variability in MTT-formazan production between wells.

The protocol described in this paper also differs from other tetrazolium-based chemosensitivity assays in that cells are exposed to drug for a defined time and then grown in drug-free medium. Although we describe a 24-h drug exposure time, it can be varied to suit alternative experimental protocols. Previous reports describe assays designed to be used in a drug-screening program where speed and ease of automation are important determinants of the assay protocol (1, 2). The assay thus included continuous drug exposure for a period of up to 11 days. In one a limit of 7 days was chosen in order to avoid the need to feed the cells during the assay (1). Thus, the drug exposure time and growth period are combined. For studies of drug sensitivity, particularly where small changes in sensitivity are critical, a growth period is essential. It allows for elimination of cells killed by the drug and distinguishes clearly between those cells which survive drug treatment but can no longer proliferate and those which continue to proliferate (1). The importance of this is apparent from a decrease in the ID50 over the first 3 days after drug exposure (Table 2). Another advantage is that differences in cell survival at different drug concentrations are amplified. This effect is maximized if the cells are fed daily, such that growth is not limited by nutrient supply.

The disadvantage of continuous drug exposure is that it takes no account of drug stability or of drug metabolism, the products of which may be either active or inactive. The half-life of some mitomycins in tissue culture medium is about 2 h and that of doxorubicin only 15 h (10). Furthermore, there are additional problems which are specific to the drug used. For example, the cardiotoxicity of doxorubicin is associated with alterations in mitochondrial enzyme activities (11). Although the exact site or sites of MTT reduction in intact cells are unclear, it is known that mitochondrial dehydrogenase activities can reduce the dye (12, 13). Thus, with continuous exposure, doxorubicin could interfere with MTT reduction as a result of nonlethal damage to the cells. Furthermore, the absorption maximum for doxorubicin is between 500 and 600 nm, and thus it may well interfere with MTT reduction at 570 nm as noted previously (1). Therefore, we use a defined drug exposure time and include a growth period of about 3 cell doubling times.

Thus, the assay described differs markedly from previously described tetrazolium dye-based assays. Cells are incubated with MTT, but the MTT concentration is predetermined for the cell line in order to ensure maximum MTT-formazan production. The formazan product is dissolved in DMSO but, in contrast to other assays, the pH is then adjusted to 10.5 so as to overcome the effects of culture medium and cell density on the MTT-formazan absorption spectrum. In addition, cells are exposed to drug for a defined time, and surviving cells are allowed to grow for two to three cell-doubling times in the absence of drug. During this time, the culture medium is replaced daily such that the growth rate is not limited by nutrient depletion. These modifications clearly detract from the advantages of the assay in terms of automation. However, we describe a rapid and simple chemosensitivity assay that gives results closely similar to those obtained by a clonogenic assay which can be used for both adherent and nonadherent cell lines.

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Effects of the pH Dependence of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide-Formazan Absorption on Chemosensitivity Determined by a Novel Tetrazolium-based Assay

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