



SERIAL DILUTION TESTING

There are a number of protocols that require serial dilutions. They are primarily used to determine the range of concentrations of one substance and its effect on another parameter. A typical protocol is IC50 testing (inhibitory concentration of 50%) of compounds in drug discovery.

Multiple dilutions of the drug are made in a diluent. Usually these are two fold serial dilutions but they can really be anything - three fold, ten fold or simply several points on a dilution curve. The test parameter is added to determine at what concentration does the compound produce the desired effect.

In High Throughput Screening there are normally a number of compounds that require an IC50 type of determination. If the number is large enough the most cost effective method is by the plate. For this example, assume there are 96 compounds that need to be tested at several concentrations. Assume the master library has compounds at a 10^{-3} concentration and it is desired to test them at 10^{-5} , 10^{-6} and then in three fold dilutions for five more places.

A Quadra 96[®] would first aspirate 90 μ L of diluent and 10 μ L of the master plate compounds. This is dispensed into plate # 1, providing a 10^{-4} dilution. 10 μ L is aspirated from this plate combined with another 90 μ L of diluent into a second plate. This plate now has one of the desired concentrations of 10^{-5} . This same technique is used to create a third plate from the second plate, which provides the next required dilution of 10^{-6} .

On the remainder of the plates a three fold dilution is required. This is accomplished by aspirating 50 μ L from plate # 3 and mixing it with 100 μ L of diluent into plate # 4. This is repeated for the number of three fold dilutions that are desired.

In the above procedure, plate-to-plate transfers were made, 96 samples at a time. This can be accomplished with a Quadra in less than a minute, per plate. The dilutions are simply "printed" from plate to plate with the desired amount of diluent. Changing the amount of compound vs. diluent changes the dilution ratio.

The other end of serial dilution testing is typified by antibiotic susceptibility testing in clinical microbiology. It is desired to know which of several drugs will inhibit growth of the patient's infective organism, and at what concentration. In this case, 100 μ L of nutritive broth is added to all wells of a 96 well plate. A single row of eight tips is loaded on the Quadra head, 100 μ L of the initial drug concentration is aspirated from the microplate containing the eight drugs to be tested. This 100 μ L of drug is mixed with the 100 μ L of diluent in the well, making a two-fold dilution. 100 μ L of this dilution is transferred and mixed with the 100 μ L of diluent in the second row, making a 4:1 dilution. This proceeds consecutively down the plate making two fold dilutions in each well. Again as before, the dilution factor is the ratio between diluent and drug transfer, this is easily controlled by the Quadra program. The factor can be easily changed on each row.

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After the dilutions of the drug are completed, an aliquot of the patient's organism is added to all wells and the plate is incubated overnight. Where the drug concentration was sufficient to kill the organism, no growth appeared and the wells will be clear. At the point there is insufficient drug to kill the organism the well will be cloudy, indicating growth. That is the minimum inhibitory concentration (MIC) of that drug against that specific organism. The overall test of several drugs indicates which is the most cost effective treatment and at what concentration must be achieved in the patient.

Serial dilution testing has many applications and can be accomplished several ways as these two examples indicate. The Quadra-Plus™ has several advantages in the automation of serial dilution testing. It can be fitted with two 96 well heads. One must be the 450µL head with polypropylene tips. This allows loading single rows of tips in either the eight or twelve direction. Actually two or more rows can be used depending on the length of serial dilution required. The other pipetting head can be the 96 small volume head. It is used to add the diluent and common reagents, after the dilution is made. These tips are limited to 60µL total volume, but multiple passes may be used if higher test volumes are desired.

The 96SV head adds diluent to the plate from the on board reservoir. The row or rows of tips are loaded onto the 450µL head by the tip jig in the normal manner. The stages under both heads on the Quadra-Plus™ are full indexing. The stage can position a row of tips anywhere in a microplate, in the X or Y axis. They first pick up the compound from the compound plate, then dispense it in the first row of the dilution plate. The serial dilution proceeds as described above. The 96SV head may then add the common reagents to the dilution plate to complete the protocol.

The combination of multiple pipetting heads, multiple stackers, 8 stations on the Quadra shuttle and the indexing stages, permit the serial dilution testing to run unattended through the supply of plates in the plate stacker cassettes.