## **Guidelines for Using Test Kits**

Conscientious aquarists often strive for perfect water quality with the use of test kits. Not infrequently, they discover that kits do not always agree or give results that are at odds with other observations. Many test kits perform well, while some defy rational possibilities. Not even the best kits, however, can perform exceptionally well at all times and under all possible conditions. This SeaGram<sup>TM</sup> should help you select kits that are generally reliable, identify conditions that will render them unreliable, and dispel some of the prevalent misconceptions.

Because of the historically bad shelf life of some of the kits of several years ago, the common notion today is that dry reagents are superior to liquid reagents. This may be true for some tests, but certainly not all. For some reagents, liquids are more stable than dry reagents. Liquids

are usually easier to use and more economical. Liquids are also less prone to precision errors. Liquid technology has improved remarkably in the past several years. Dry powder

reagents are less likely to have problems than compressed dry reagents (tablets). The choice between liquids and dry reagents should be made on a case by case basis. Colorimeters are too expensive for most aquarists, so almost all kits rely on visual color matching. Because of this, only those kits should be selected that have unambiguous color differences in the practical measuring range. Transparent colors are difficult to match against opaque colors, so a kit should use the same kind of reference scale as is produced by the test. Ideally, color differences should reflect both a hue and intensity gradation. Kits that produce only an intensity difference are quite difficult to read reliably. The human eye is better capable of differentiating variations in reflected colors than transparent colors. Kits should contain a reference sample which can be used to both learn how to use the kit properly and check the integrity of the reagents. In the aquarium industry, Seachem alone includes a reference sample with its kits.

pH kits should have a sharp color change over a narrow range of pH. Avoid kits that use insensitive single color dyes such as phenol red or cresol red. The better kits usually go from yellow to green to blue, or some variation of that. Color scales should be specific for fresh or marine water, since salts have a pronounced effect on dye response to pH. Most pH dyes are very stable, so there is no inherent need for dry reagents. Water containing proteins or polymers will generally give false low results because these polymers rapidly bind to pH dyes. Weakly buffered water will give unreliable results, because the dyes themselves have an internal pH that must be overcome by the sample. Imprecision in the amount of reagent or sample used can also cause significant errors. Strong reducing agents, as are contained in dechlorinators and ammonia conditioners,

may cause fading of color. Seachem's

MultiTest®: Marine pH & Alkalinity kit uses a very narrow range indicator which goes from colorless at 7.6 to blue-black at 8.6, being a clear sky blue at pH 8.3 only. The dye is also

relatively insensitive to salt and protein effects. The kit includes a titrating agent so that alkalinity may be determined with the same kit. As with other MultiTests<sup>TM</sup>, the tests are read from an opaque white test plate, which permits easy reading and comparisons of several samples at the same time.

Ammonia kits fall into three groups: Nessler's reagent (yellow with ammonia); salicylate-hypochlorite (green to blue with ammonia); and hydrophobic sensors, Seachem's Ammonia Alert™ and MultiTest®: Free & Total Ammonia. Nessler's reagent is a highly toxic mercury complex and should not be used. Nessler's reagent is also very susceptible to interference from common aquarium products. Since both Nessler and salicylate tests are run at a pH of greater than 12, they measure as ammonia any substance which can generate ammonia or primary amine groups at high pH, including ammonia complexed to ammonia removing conditioners. The salicylate tests rely

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on the reaction of ammonia with hypochlorite or chlorine, and, consequently, any aquarium product capable of removing chlorine (dechlorinators and bisulfite based ammonia conditioners) can be expected to interfere with color development, even when ammonia is present. The latter is a convenient coincidence for some ammonia removal products, since it gives the appearance that the ammonia conditioner is performing much better than it actually may be. The hydrophobic sensor technology relies on the vapor phase of free ammonia and is, consequently, more specific for ammonia and less likely to be affected by contaminants in the sample. It has the distinct advantage of being able to differentiate free ammonia. Total ammonia can be measured by raising the sample pH. These sensors can measure true ammonia, even in the presence of ammonia conditioners. This technology is available exclusively from Seachem in its Ammonia Alert™ and MultiTest®: Free & Total Ammonia kit.

Nitrate is usually quantified by reducing it to nitrite, which is then detected by the formation of a diazonium dye. Nitrite is measured directly by the same diazonium dye. Chemically, all these test kits measure the same thing regardless of the units used, be it reported as nitrogen, nitrate, or nitrite. Test kits differ on the specific diazonium dve formed and this is reflected in the intensity of color produced and thus the sensitivity and range of the kits. For aquarium usage, test kits with low range sensitivity are more useful. In general, nitrite tests are not susceptible to interference from common aquarium products. It is possible that strong reducing agents (e.g., bisulfite based ammonia conditioners) could either yield low results by bleaching out color formed or yield high results by reducing nitrates to nitrites. Likewise, strong oxidants may interfere with the formation of the diazonium dye. Seachem's MultiTest®: Nitrite/Nitrate uses a six well multiple test plate to measure up to six samples at the same time. The kit is designed to function in the low range, detecting as little as 0.1 mg/L nitrite or 0.2 mg/L nitrate.

Phosphate can cause problems in reef aquaria by interfering with the physiology of corals. Although less critical in the non-reef aquarium, phosphate can also cause problems in other aquaria that are related to overgrowth of algae. Phosphate kits are based on the reaction of phosphate with molybdic acid to form a phosphatemolybdenum polymer, yielding either a yellow or blue color. This reaction, however, is quite insensitive; consequently, low range kits tend to be cumbersome and expensive because of design requirements to increase the sensitivity. Phosphate testing is also prone to falsely elevated results from acid neutralization caused by high alkalinities. Seachem's MultiTest®: Phosphate kit uses a unique catalyst that permits high sensitivity within the simple and easy to use design parameters of the multiple test plate approach. MultiTest®: Phosphate is sensitive to less than 0.05 mg/L phosphate and yields clear colors from yellow to green. As with other MultiTest  $^{\scriptscriptstyle \mathsf{TM}}$  kits, the Seachem kit is unique in including a reference sample for test validation. Seachem also has a related test, MultiTest(R): Silicate, which resolves less than 0.5 mg/L silicate.

Because marine water contains almost five times more magnesium than calcium, and also contains strontium, two elements that are very similar to calcium, calcium kits have problems when used with marine aguarium water samples. Aquarium water also is likely to contain significant quantities of amino acids, proteins, and other chelating agents that interfere with compleximetric dyes and titrants used to measure calcium. Another problem arises from the very narrow range of concentration to be measured with calcium. For pH, each unit change represents a 10-fold difference in concentration. For ammonia, nitrite, nitrate, and even copper, the range of interest is from 0.1 to 0.2 or more units, a two-fold (100%) concentration difference at the least. But with calcium, even a difference between 350 mg/L and 400 mg/L represents only a 12%-15% difference, which is much more difficult to resolve. Seachem has a calcium test kit specifically designed for aquarium use, Reef Status:<sup>TM</sup> Calcium, which readily and easily measures calcium in both marine and fresh water with a resolution of less than 5 mg/L. Seachem also offers a kit for measuring strontium, Reef Status: TM Strontium, with a resolution of 0.50 mg/L. Magnesium can be monitored as well with our Reef Status:™ Magnesium, Carbonate & Borate to a resolution of 12.5 mg/L. Seachem's MultiTest®: Iodine/ Iodide represents an additional test for primarily reef aquariums previously unavailable to the hobby. The innovative MultiTest®: Iodine/Iodide kit offers a quantitative test for this important element of soft and hard coral growth.

Copper kits are of two types: compleximetric or titration based. Some kits measure only ionic copper and others measure both ionic and chelated copper. Seachem's MultiTest®: Copper measures both types of copper to a resolution of better than 0.05 mg/L and uses a sensitive catalytic compleximetric chemistry. For the planted aquarium, Seachem has MultiTest®: Iron, which rapidly measures iron to less than 0.02 mg/L.

Problems with any test kit originate either from loss of reagent integrity or interfering substances in the aquarium water. A check of reagent integrity is easily done if the kit includes a reference sample, as do Seachem kits. Analytical reagents, dry or liquid, tend to be adversely altered by light and heat. It is a good idea to store kits in a cool, dark place. Problems with the sample are a little more elusive. One good practice is to add known amounts of what is to be measured to a known amount of aquarium water to determine if what has been added can be measured in the quantities added. For example, if one adds 100 mg of calcium to a liter of marine aquarium water and the same to a liter of freshly prepared saltwater, and a calcium kit indicates a 100 mg increase in the freshly prepared water but not in the aquarium water, that is a reliable indication that something is present in the aquarium that interferes with the kit reagents. Likely sources of interference are anything that is chemically reactive. Examples include added or endogenous chelating agents, such as amino acids, proteins, or dibasic acids; reducing agents or antioxidants such as dechlorinators, ammonia removers, vitamin C, or fatty acids; oxidizers such as ozone, peroxide, permanganate, or persulfate; and high redox.