

Discussion and Recommendations pertaining to the use of the Abraxis ELISA “PSP Shipboard kit” for PSP analyses

Holding time for extracted samples

Although the Abraxis ELISA kit will specify that extracted samples using the “rapid extraction method” (isopropyl alcohol and distilled vinegar), may be frozen for up to a week, we conducted an informal study on several samples using “old” extracts (held for four days at -20 degrees), and compared them to fresh extractions from the same homogenates, prepared on the day of the testing. Of the three samples in this small study, two samples which were below the detectable limits as “fresh” extracts showed low-level toxin scores (26 and 30) after four days of storage in the freezer, and the third sample showed a 19% increase in the toxicity score in the “old” extract used, when compared to the “fresh” extract. Although three samples is not a large enough set to make any statistical determinations, the data does suggest that running “old” extracts through the Abraxis kit will result in slightly higher final toxicity scores. It is recommended that the user extract and prepare only those samples which will be analyzed that day, and leave any additional samples in homogenate form in the freezer until there is time to both extract and analyze the samples on the same day.

Accessory Pack - dilution of samples

The Abraxis “Accessory Pack” was used to prepare extracted homogenate samples for analyses in the ELISA kit. The Accessory Pack contains a series of small glass vials which are pre-filled with the diluent needed for this purpose. One Accessory Pack contains enough vials to dilute 20 samples for testing; however, it is recommended that the user have additional Accessory Packs on hand, beyond the exact number needed for testing purposes, since several of the pre-filled vials were found to be either broken, or had otherwise lost some of the pre-measured volume of diluent.

Operator variability – R² and %CV values

The Abraxis ELISA kit is sensitive to operator error and variability, which can be observed at the end of the test in the calculated R² and %CV values. Even an experienced test operator should plan on “breaking themselves in” on a practice round before going on to test results that will be recorded as final. For example, despite the fact that I have run hundreds of Abraxis tests, my initial R² value on the first round was 0.9127. The user should strive to achieve a R² value which is >0.97 in order to consider the results valid and reportable. It is recommended that additional testing supplies be allocated above and beyond the exact number needed for samples, in order to accommodate this “practice run” for the operator.

The %CV values are also reported out in the final spreadsheet, and operator error will sometimes result in unusually high %CV values. Any samples which result in a calculated CV value that is ≥ 10% should be re-tested on a fresh plate. You will note that in the raw ELISA results that have been included with this packet, there were several samples for which the initial CV was > 10%, which are highlighted with a yellow bar; these samples were re-run on another plate, resulting in acceptable %CV values, and those results are the ones recorded in the final report.

Recommended maximum plate load

The Abraxis kit comes with a 96-well plate, which is technically capable of running as many as 26 discreet samples with standards, in triplicate; however, the “Shipboard kit” version is designed to be used with a hand-held photometer, which only allows for readings on one well at a time. Since the final step in the assay, which involves reading the wells with the photometer, must be completed within 15 minutes in order to obtain accurate results, it is recommended that the operator only screen up to 10 discreet samples in one run. This will result in a total yield of only 20 possible samples per plate, but it will result in more accurate results, and is worth the additional cost associated with budgeting only 20 samples per kit.

Comparison between Abraxis and MBA results

The final results from the NH homogenate testing compare Abraxis ELISA results with the Mouse Bioassay (MBA) results provided by the state of New Hampshire. Unfortunately, there is not a

consistent, linear relationship between the two methods; in some cases, the Abraxis results were reporting a higher value than the MBA results, while in other cases, the Abraxis results were reporting lower than the MBA results. This is something that a resource manager should be thinking over long and hard, before committing to a complete transition to Abraxis testing as a replacement for the MBA, however, there was an overall pattern in the sample results that could be used to reduce the number of MBA tests performed over the course of a season. In the early season, from April through early May, the MBA testing was showing all negative results, while the Abraxis kit was showing results ranging from 24 – 34 ug stx eq / 100g during this same early season time frame; a manager could potentially consider using the Abraxis kit to screen early season results and forgo the use of the MBA until Abraxis results approach 35 or higher, saving on resource allocation for mouse testing, while still keeping a comfortable margin for protecting public health. Due to the unusual results reported from early May through mid-June, during which time there were at least three cases where the MBA results were higher than the Abraxis results, at one point nearly reaching the FDA quarantine limit, then it is not recommended that the Abraxis kit be used during an active, ongoing PSP bloom in lieu of the MBA, but additional collection of comparable data over time may be useful.