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Crocodile miniWorkstation

Diagnostic Sensitivity and Specificity of samples tested with the Crocodile miniWorkstation and Bovine Serum Albumin ELISA Kit from Sedium R&D

Introduction:

An ELISA protocol contains typical routine steps like the addition of different reagents or incubations, microplate washing and OD-measurement. The usage of physically large instruments or the requirement for multiple instruments to perform assay functions leads to a crowded and cluttered work area that decreases productivity. The new CrocodileminiWorkstation provides the same functionality as five individual instruments in a footprint only slightly larger than a standard stand alone reader. This note will show the diagnostic sensitivity and specificity of the system using the ELISA test Bovine Serum Albumin ELISA kit (Sedium R&D).

Beef is a significant component of food for a considerable part of the word population. The prevalence of allergies to BSA in no doubt is less than that to other food allergens. However allergologists do recommend the determination of BSA as a foodstuff allergen. Not negligible is also the fact that the presence or absence of BSA in foodstuff can be exploited as the parameter utilisable for assessment/evaluation of the specified foodstuff composition as well as of observance of the produce specified for the foodstuff production.

Description:
Bovine Serum Album ELISA kit is a competitive immunoassay for the determination of the bovine serum album (BSA) in the food samples. The kit results are always negative for food matrices which naturally do not contain BSA.

Materials:

Instrumentation:	Crocodile miniWorkstation
	Single channel pipette (20-200 µl)
Reagents:	Bovine Serum Albumin ELISA Kit Cat.No.: FA 00308/48; Lot 004;
	exp DateAugust 31th 2011
	Demineralized water
Consumables	Solution reservoirs
	Pipette tips

Method:

Definitions and test procedure:

- Reliability (Test-retest reliability) is the variation in measurements taken by a single person or instrument on the same item and under the same conditions. For this purpose, four standard and two control samples were tested in three independent runs as duplicates to determine the CV%.
- Reproducibility is defined as the ability to independently reproduce results e.g. by testing in different plates on different days. For this purpose 21 samples were tested in two independent runs to determine the correlation coefficient “r”and the coefficient of determination “r^2”.

- Diagnostic sensitivity is defined as the ability to correctly identify BSA contaminated samples whereas diagnostic specificity is defined as the ability to correctly identify non-contaminated samples. To determine diagnostic sensitivity and specificity 21 samples were analysed using the Crocodile miniWorkstation. 9 of the used samples were spiked with BSA and 12 samples were confirmed negative samples. The limit of qualification for this test kit was described at 3,76 ppm.

- Reagent and Sample dilution was performed as described in the test procedure document. Controls and samples were determined in duplicates.

Assay principle:

In the wells of a microtiter plate, walls of which are coated with sheep anti-BSA antibody, the sample to be analysed, while combined with BSA conjugate, is homogenized with horse – radish peroxidase. During the step of incubation, BSA will be bound to the wells walls. BSA present in the sample and BSA bound in the conjugate will compete mutually for access to the binding sites present in limited number in the antibody against BSA. After a subsequent incubation step, the wells are washed and the peroxidase bound to the wells walls is detected by means of a chromogenic substrate (TMB), added to the system. Intensity of thus development coloration is inversely proportional to the BSA concentration in calibrators and analysed samples.

Reagent and Sample dilutions were performed as described in the test procedure document.

Results:

- Reliability:

The standard deviation (SD) is the way of describing how dispersed a set of values is from the mean. The coefficient of variation (CV) is defined as the ratio of standard deviation to the mean. The CV is a standardization of the SD that allows comparison of variability of an assay.The inter-assay variance of the kit is described with 13,6% CV.

Sample	OD	OD	CV%
STD0	2,095	1,973	3,0
STD1	1,36	1,249	4,3
STD2	0,854	0,813	2,5
STD3	0,578	0,466	10,7
STD4	0,261	0,245	3,2

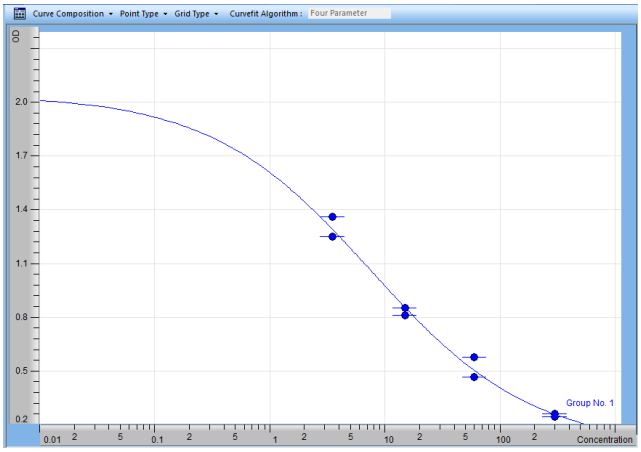
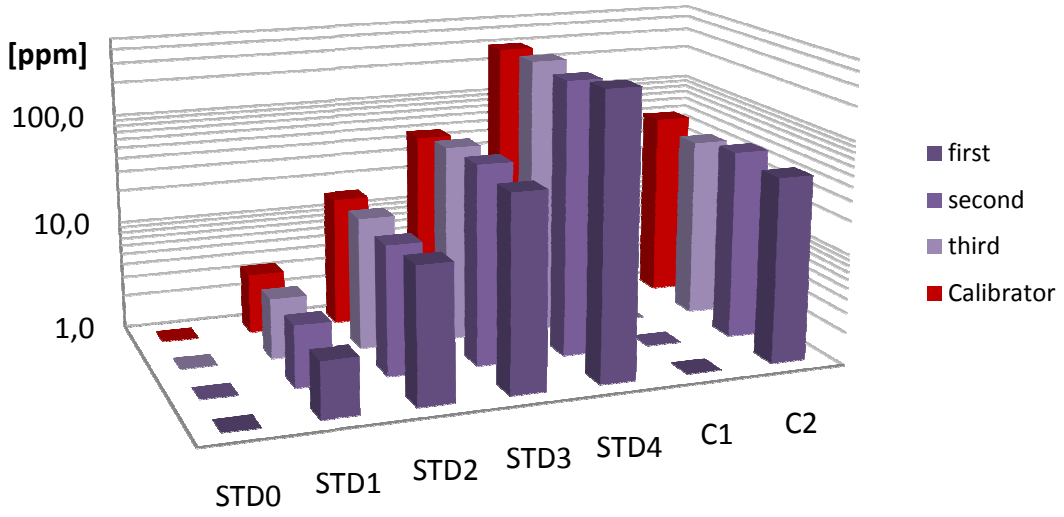


Figure 1: Standards were determined in duplicates. OD is OD_{450}. The table shows the results for one run and the CV% for each sample. The diagram shows the related standard curve with Four Parameter Curvefit Algorithm using MicoWin 2010.



OD	first	second	third	Calibrator	CV%
STD0	0,0	0,0	0,0	0,0	0,0
STD1	3,2	3,6	3,5	3,5	6,6
STD2	16,4	14,7	15,7	15,0	5,5
STD3	56,1	63,2	59,4	50,0	6,0
STD4	353,4	296,2	317,0	300,0	9,0
C1	0,0	0,0	0,0	0,0	0,0
C2	46,5	51,9	43,1	50,0	9,4

Figure 2: Table and graph of the mean values of the calculated concentration from standard and control samples of 3 independent runs.

❑ Validation criteria for reproducibility:

The linear correlation coefficient “r” measures the strength and the direction of a linear relationship between two measurements. A correlation between two independent testsgreater than 0,8is generally described as strong whereas a correlation less than 0,5 is generally described as weak.

The coefficient of determination “r^2” denotes the strength of the linear association between two tests. This coefficient is a measure of how well a regression line represents the percentage of the data that is closest to the line of the best fit.

A perfect correlation between two measurements would be indicated with an $r=1$ and $r^2=1$.

❑ Validation criteria for diagnostic sensitivity and specificity:

Diagnostic sensitivity is defined as the ability to correctly identify contaminated samples, whereasdiagnostic specificity is defined as the ability to correctly identify non-contaminatedsamples.

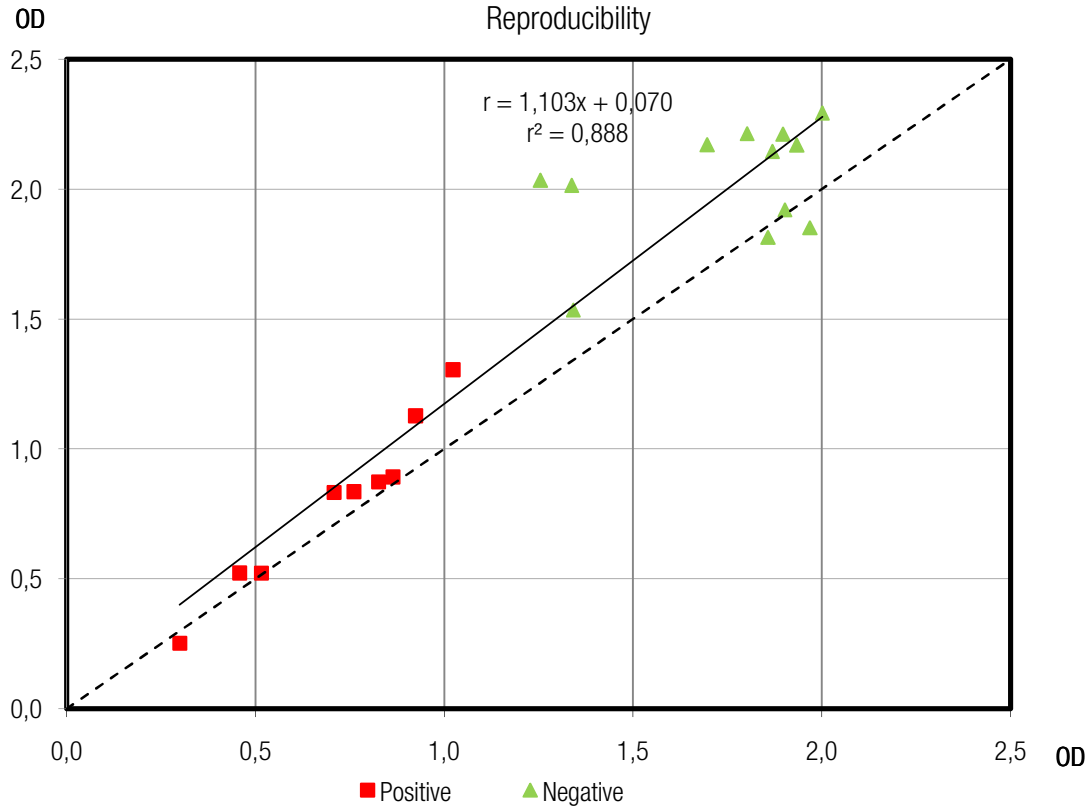


Figure 3: The graph shows the relation between the OD_{450} values of two independent tests containing results of 9 positive and 12 negative samples (samples with less than 3,76 ppm BSA concentration). The linear correlation coefficient "r" (r= 1,103x + 0,07) measures the strength and the direction of a linear relationship between both measurements. The coefficient of determination "r²" (r^2 = 0,888) denotes the strength ofthe linear association between both tests.

Summary:

To test the reliability of the assay processed by the Crocodile miniWorkstation,four standard and two control samples were tested in three independent runs in duplicates. The concentration was determined by the mean value of each data pair. The CV% of the resulting concentration for each sampleof the three runs was calculated and compared with the nominal concentration. The inter-assay variance of the kit is described with 13,6% CV

To test the reproducibility of an assay, 21 samples were tested in two independent runs with the BSA ELISA kit from Sedium R&D. The linear correlation coefficient "r" was determined with r= 1,103x + 0,07 and the coefficient of determination "r²" with r^2 = 0,888.

To test the diagnostic sensitivity and specificity of an assay processed by the Crocodile miniWorkstation, 9 positive (more than 3,76 ppm BSA) and 12 negative samples were tested in two independent runs with the BSA ELISA kit from Sedium R&D.

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Conclusions:

Using the Crocodile for the assay procedure is extremely simple and involves only the addition of the samples.

TheCrocodile miniWorkstation is excellent suitable for the performance of the BSA ELISA test. This Application note demonstrates, that reliability, reproducibility and diagnostic sensitivity and specificity of the kit was fully achieved using the Crocodile miniWorkstation.

The test-retest reliability, determined with three independent measurements showed good CV%s in the range between 5,5 and 9,4.The determined concentrations have been very close to the estimated values.

The Crocodile miniWorkstation achieved a good reproducibility in OD450 measurements. This is demonstrated by the resulting correlation coefficient on “r” with r = 1,103x + 0,07 and the coefficient of determination “r²”with r² = 0,888. A correlation between two independent tests greater than r = 0,8 is generally described as strong.

All samples with more than 3,76 ppm BSA were identified correctly as positive, showing the diagnostic sensitivity by using the combination Crocodile miniWorkstation and ELISA BSA kit from Sedium R&D.

Samples with less than 3,76 ppm BSA were identified correctly as negative, showing the diagnostic specificity by using the combination Crocodile miniWorkstation and ELISA BSA kit from Sedium R&D.

Acknowledgement:

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www.sedium-rd.cz/