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<b>MEDWISS Analytic GmbH</b>	<b>AllergyScreen/ AlleisaScreen</b>	Date 15.12.2016

## **Circular letter 12: Dilution effects on the reactive solutions by remaining washing buffer in the test troughs**

### **Background and Procedure**

The aim of this review is to examine, if remaining washing buffer in the reaction troughs of AllergyScreen/AlleisaScreen, because of an insufficient discard after the washing steps, has an effect on the results, the intensity of the control line and on the background.

Therefore, two different sera (PS and Ste) were chosen and two test strips were worked-off per condition. To imitate an incomplete emptying of the troughs after washing, 100 µl of washing buffer were pipetted into the troughs additionally to detector antibody, streptavidin-conjugate and substrate, respectively. For comparison two troughs were processed as usual in the same run – without imitating dilution of solutions by remaining washing buffer. Visual evaluation was performed after drying the membranes to analyse, if residual washing buffer has an effect on the background of the worked-off membranes. Subsequently, test strips were scanned. The sum of allergy classes and the mean values were calculated.

### **Material**

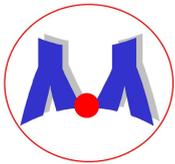
Membrane: P30 Food Iraq M-150526

Solutions: D-161107, S-161104, F-12342151, W-160609

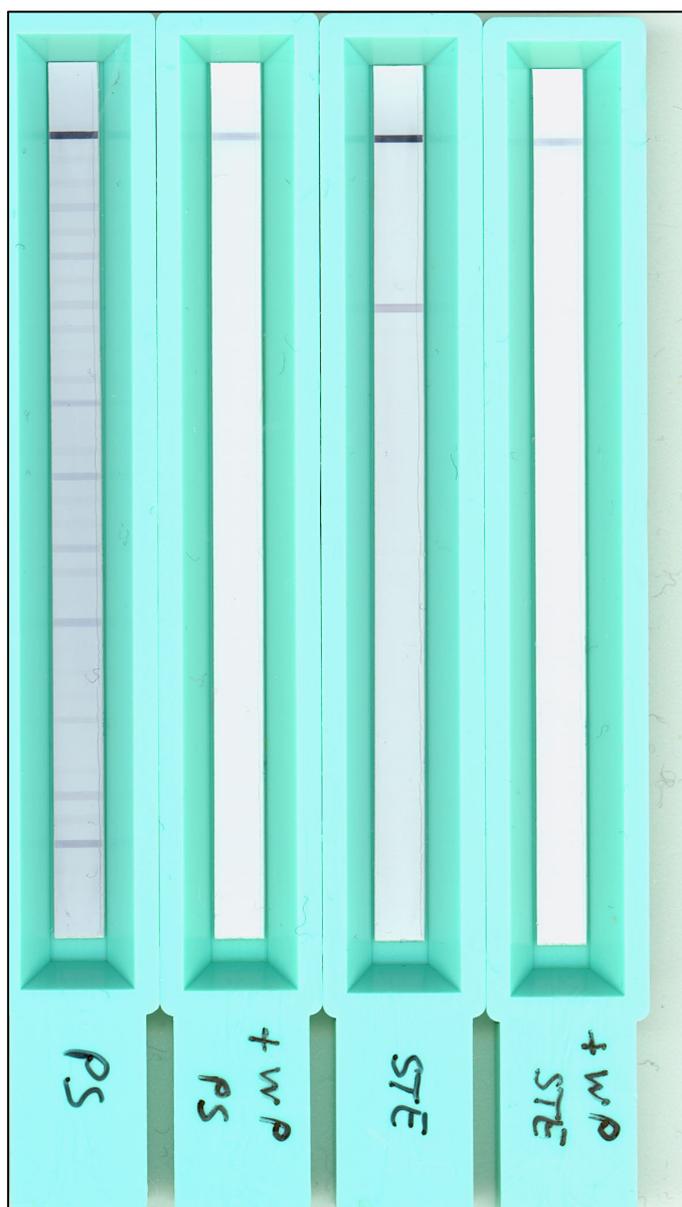
Serum: PS, STE

### **Results/ Observations**

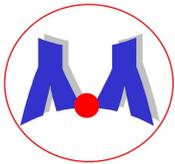
The reference strip processed with PS serum shows many positive allergen lines with values of up to class 3.2 (test strip PS; Fig. 1). The value of the control line is class 6 as expected. When solutions were diluted with washing buffer (test strip +WP PS; Fig. 1) no positive allergen lines were detected. The value of the control line is class

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3.0. The reference strip processed with Ste serum shows one positive allergen line (class 3.2). The value of the control line is class 6. Dilution of solutions with washing buffer leads to a complete extinction of the positive line. The value of the control line is reduced to class 0.1.



**Fig.1:** Membranes were processed with and without dilution of solutions with washing buffer to imitate residual washing buffer in the troughs. From the left to the right: PS: reference test strip processed with serum PS; PS +WP: test strip processed with serum PS with washing buffer treatment during processing; STE: reference test strip processed with serum Ste; STE +WP: test strip processed with serum STE with washing buffer treatment during processing.

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**Tab.1:** Values of the control lines and mean values of the sum of allergy lines from the processed test strips (see Fig. 1)

Strip	Control line (class)	Sum of allergy lines (class)
PS	6,0	34,4
PS + washing buffer	3,0	0,0
STE	6,0	3,2
STE + washing buffer	3,1	0,1

#### Background Analysis:

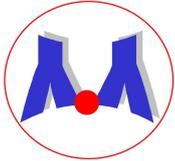
The background of the test strips treated with washing buffer is much brighter compared to the background of the reference strips. This is caused by a significant dilution of the solutions by remaining washing buffer in the test troughs.

#### **Conclusion**

The results show a strong effect caused by the remaining washing buffer in the troughs after the washing steps because of a dilution effect of the respective used reagent solution. Detection antibody, streptavidin-conjugate and substrate have a system adapted concentration, which is set very low but provides enough reactive molecules for the process. Due to the dilution with washing buffer there is an unwanted additional dilution effect of these molecules and thus they are no longer or not in a sufficient concentration, respectively, available for the primary reaction on the membrane. Values of the control line were reduced by half in this experiment. And positive allergen lines of the reference strip were completely negative when solutions are diluted with washing buffer to simulate remaining washing buffer in the troughs.

Because of this extreme effect it is very important to adhere to the instructions of use for AllergyScreen/AlleisaScreen concerning the washing steps.

On the one hand washing has to be performed thoroughly. Otherwise the membranes can get a blue background after drying which can lead to too low results.

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**On the other hand it is important to discard and remove the washing buffer adequate after each washing step. If there is still washing buffer in the trough because of an insufficient emptying and a solution is pipetted in the trough, there will be a dilution effect which can lead to too low and even false negative results, respectively.**

See also: abstract of the instruction of use for the AllergyScreen/AlleisaScreen regarding the washing and the discard of the washing buffer after the washing steps: ..., **beat the troughs gently on paper towels or filter paper to remove surplus washing buffer.**