# Detection of gluten on surfaces and cleaning-in-place waters using the R5 dip stick

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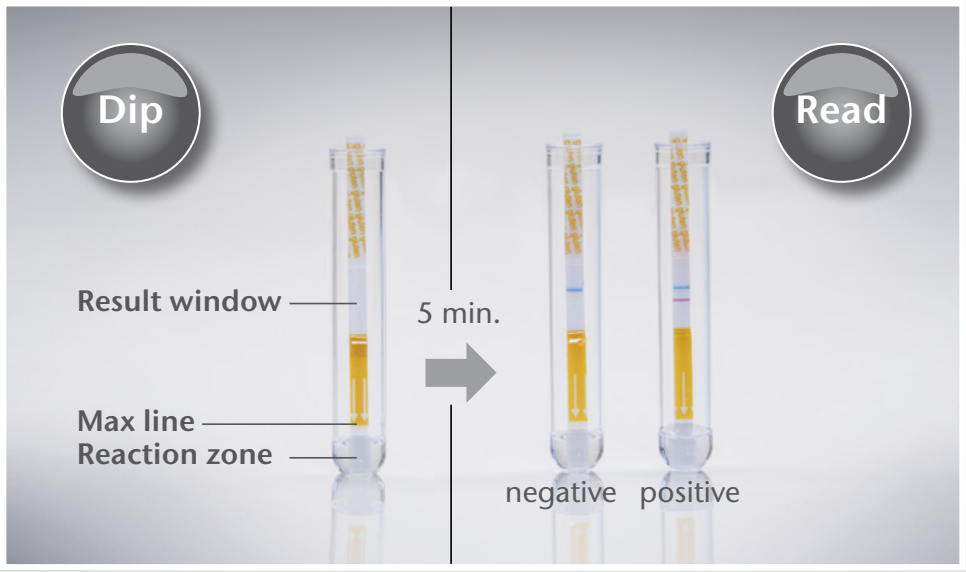
## Introduction

In 2015, the AOAC Gluten Expert Review Panel adopted the dip-stick RIDA®QUICK Gliadin (R-Biopharm, R7003) as AOAC *Official Method*™ 2015.16 First Action for processed and non-processed food. Since the dip-stick is also suitable for direct swabbing procedures, several surfaces and additionally cleaning-in-place (CIP) waters (with and without surfactants) have been tested as part of the AOAC *Performance Tested Method*™ process.

**Picture 1:** Schematic presentation of the test principle and the subsequent interpretation of the possible results (invalid results not shown)




## Methods

All surfaces (stainless steel, plastic, silicone rubber, and sealed ceramic) were contaminated with a PWG-gliadin solution at levels of 0, 0.25, 0.5, 1.0, 2.0, and 4.0 µg/100 $cm^2$ gliadin. After drying, 10 x 10 cm surface areas were swabbed directly with the dip-stick. Afterwards the stick was incubated for 5 min in 500 µl buffer and read out visually for a positive or negative result (figure 1). 20 replicates were tested for each concentration.

CIP waters were spiked with a commercial gluten preparation (G5004, Sigma-Aldrich) at levels of 0, 25, 50, 100, and 200 ng/ml gluten. 50 µl of CIP waters were pipeted to 500 µl of buffer and a dip-stick was added. After 5 min of incubation, the dip-stick was read out visually for a positive or negative result (Figure 1). 20 replicates were tested for each concentration.

Pure water without any cleansing reagent was spiked with a commercial gluten preparation (G5004, Sigma-Aldrich) at levels of 0, 4.5, 9.1, 18.2 and 36.4 ng/ml gluten. 225 µl of water were pipeted to 225 µl of buffer and a dip-stick was added. After 5 min of incubation, the dip-stick was read out visually for a positive or negative result (Figure 1). 20 replicates were tested for each concentration.

Surface and CIP waters testings were blind coded since contamination of surfaces and CIP waters on one hand and swabbing and testing on the other hand were performed separately by different analysts. Probability of detection (POD) curves were constructed and used to estimate an LOD 95 % gluten amount.

## Results

The results for surfaces are given in table 1 as probabilities of detection (POD), which is the percentage of positive results out of 20 replicates. Only 2 positive results out of 80 replicates for all surfaces in total were observed for non-contaminated surfaces.

The concentration range with negative and positive results was between 0.5 µg and 2.0 µg/100 $cm^2$ gluten. Starting with 4 µg/100 $cm^2$ gluten), all results were positive. Using a 4-parameter curve fitting LOD 95 % concentrations were also estimated (table 1).

**Table 1:** Results for testing gluten-contaminated surfaces with an area of 10 x 10 cm for each swabbing experiment; 20 replicates per level and surface

| | Surface (probability of detection) | | | |
|---|---|---|---|---|
| **µg/100 $cm^2$ gluten** | Ceramic tiles | Silicone rubber | Plastic (petri dish) | Stainless steel |
| 0.0 | 0.05 | 0.00 | 0.05 | 0.00 |
| 0.5 | 0.35 | 0.25 | 0.5 | 0.25 |
| 1.0 | 0.65 | 0.80 | 0.85 | 0.25 |
| 2.0 | 0.90 | 1.0 | 0.95 | 0.80 |
| 4.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 8.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| **LOD 95 % (µg/100 $cm^2$)** | **2.8** | **1.6** | **1.6** | **1.5** |

Figure 2 shows the POD response curve for the cleansing reagent Micro Quat Classic, which was used to evaluate the LOD 95 % concentration. Using a 4-parameter curve fitting an LOD 95 % concentration of 66 ng/mL gluten was estimated. Other cleansing reagents (Acifoam VF10 and Divosan Extra VT55) gave comparable LOD results of (results not shown). Analysis of pure water resulted - due to the lower dilution factor (2 instead of 11 for cleansing reagents) - in an approx. 5 times lower LOD (data not shown). Hypochlorite containing cleansing reagents (e.g. Hypofoam VF6) destroyed gluten so that it was not detectable any longer.

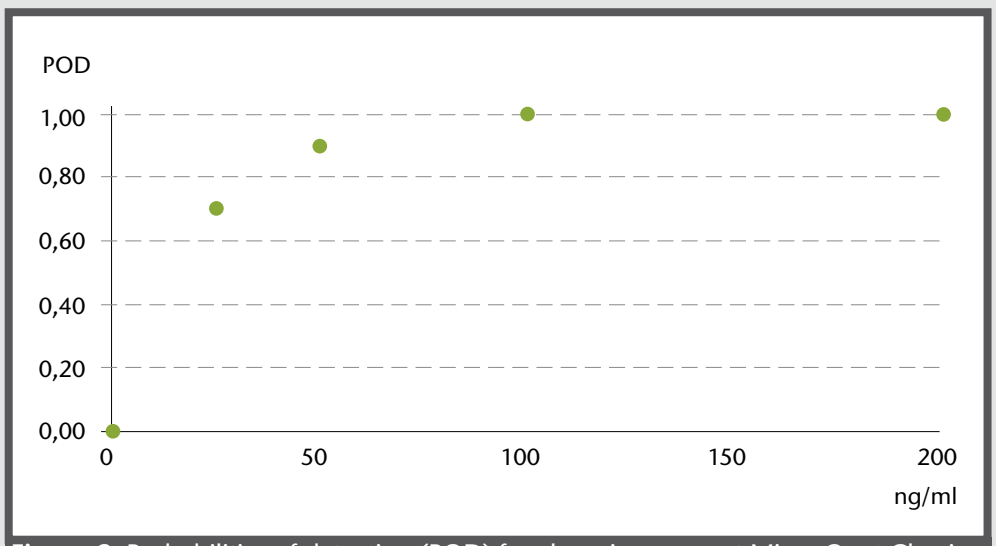



**Figure 2:** Probabilities of detection (POD) for cleansing reagent Micro Quat Classic; 20 replicates per gluten level

Results of this validation study were independently confirmed at an external lab using a gluten preparation for contamination. Matrices were stainless steel and CIP water containing the cleansing reagent Micro-Quat Classic (results not shown). This proves the excellent suitability of the RIDA®QUICK Gliadin not only for food analysis but also for surface and CIP water testing.

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