NEOGEN® CORPORATION

Lansing, Michigan USA

# The Development and Validation of Reveal® 3-D Total Milk Allergen Test, a Lateral Flow Device for the Rapid Detection of Total Milk Proteins in Clean-in-Place Rinse (CIP) and Environmental Samples

**David Almy, Emily Slenk, Frank Klein and Jennifer Rice**



## ABSTRACT

A rapid lateral flow device for detection of total milk protein (including casein and whey) has been developed. This is a sandwich-based enzyme-linked immunosorbent assay (S-ELISA) utilizing one antibody for capture and a second antibody linked to a colored particle for detection. An extraction process has been developed to allow for detection of milk protein in various sample types, including CIP and environmental samples. Reveal 3-D Total Milk Allergen Test is an accurate, rapid and simple assay that requires no special tools or extensive training and yields results in less than ten minutes from sample collection. The lower limit of detection is 5 ppm in CIP solutions and 20µg/100 cm² for total milk proteins. Importantly, Reveal 3-D Total Milk Allergen Test lateral flow device has a third line of detection, which is included to signal the presence of a grossly contaminated solution or surface and eliminate potential for false negative results in these samples.

**Purpose:** The purpose of this study was to develop and validate Reveal 3-D Total Milk Allergen Test for detection of milk proteins in CIP solutions and environmental samples and to determine the limit of detection of this assay.


## INTRODUCTION

Allergy to bovine milk proteins represents one of the most common food-based allergies, however, cow's milk is one of the most common ingredients in food and nutritional supplements. Because of the widespread use of milk and milk proteins in food production, there is a need to screen for the presence of milk proteins in food handling areas and equipment. Reveal 3-D Total Milk Allergen Test is a rapid lateral flow device which provides rapid and accurate information regarding presence of milk proteins.

The assay run time is 6 minutes which includes a 1 minute extraction and 5 minute device run time.

When analyzing rinses or liquid products, milk residues are detectable at a level of 5–10 ppm. Levels of 20 µg/100 cm² of milk (casein or whey) on environmental surfaces can be detected.

This poster will report assay performance, validation and beta site test results.

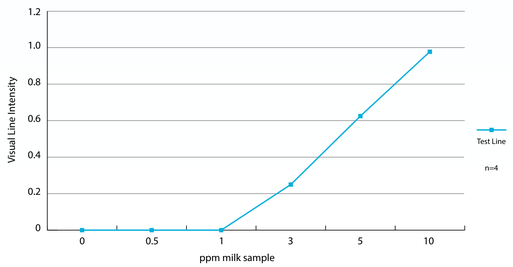
### SCORING AND INTERPRETING LINE INTENSITIES

Throughout this testing, the line intensity of the control, test and overload line was scored by comparing the device to a reference card. The scale is measured between 0 (no line intensity) – 5 (the highest line intensity).

### LIMIT OF DETECTION

The limit of detection (LOD) was determined to be 5 ppm by evaluating levels of milk protein from 0–10 ppm in 24 milk spiked buffer samples. These samples were treated as rinse samples and follow the kit instructions for such a sample.

### LIMIT OF DETECTION DATA




## Reveal® 3-D Total Milk Procedure

**Reveal® 3-D**
**Total Milk Allergen Test**

### RINSE SAMPLING

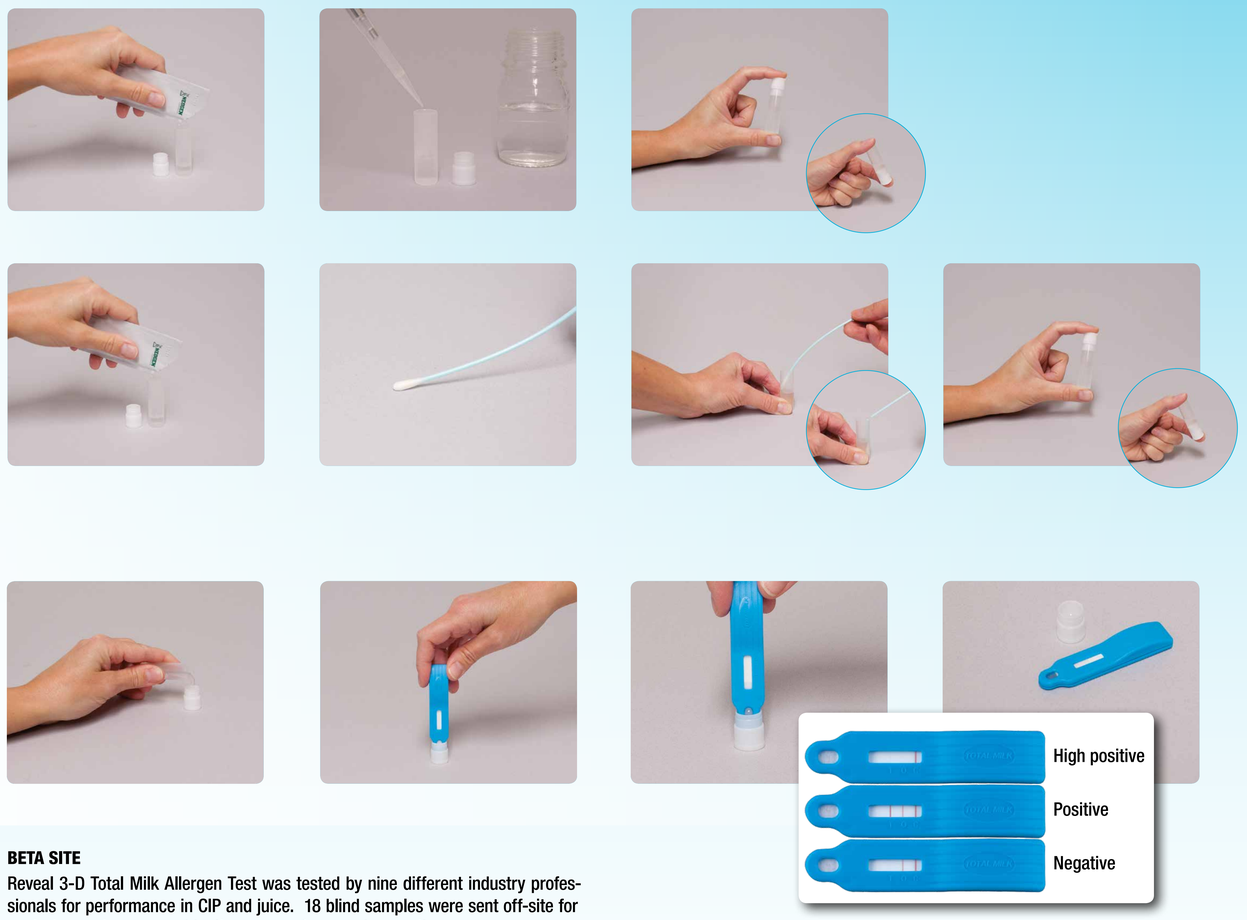
1. Open Type 8 extraction buffer sachet and add to a sample tube.
2. Add 1 mL of sample to the sample tube.
3. Secure cap and shake for 1 minute.

### SWAB SAMPLING

1. Open Type 8 extraction buffer sachet and add to a sample tube.
2. Gather the sample. *For dry surfaces moisten with extraction solution. Do not pre-moisten for wet surfaces.*
3. Return the swab to the extraction buffer and break off into the tube.
4. Secure cap and shake for 1 minute.

### SAMPLE TESTING

1. Remove the lid and fill with liquid from the tube.
2. Dip the Reveal 3-D device into the liquid in the lid. Ensure that the cavity is saturated with the liquid.
3. Leave the cavity saturated until the liquid is observed into the test window.
4. Place the device on a flate surface and allow the test to develop for 5 minutes.
5. Interpret the results.

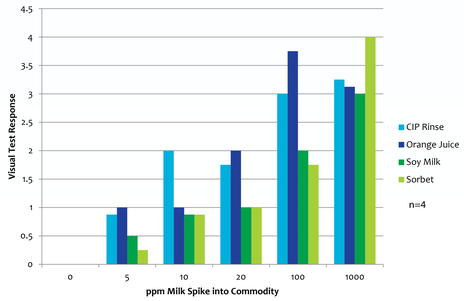



### CIP RINSE AND COMMODITY TESTING

In this situation, 24 samples of CIP rinse, orange juice, soy milk and sorbet were tested to determine recovery of total milk protein. A stock solution of milk at a known level was spiked into the various non-detect commodities at the desired testing levels. These were treated as rinse samples and tested as such. The extraction volume for sorbet samples must be altered to reduce matrix effects (0.25 mL sample volume).

At 0 ppm milk, all samples produced a negative result. A visually positive result was observed at 5 ppm milk in CIP rinses, orange juice and soy milk. In sorbet samples the limit of detection was 10 ppm milk. The overload and control lines performed as expected.

### CIP RINSE AND COMMODITY RESULTS




### BETA SITE

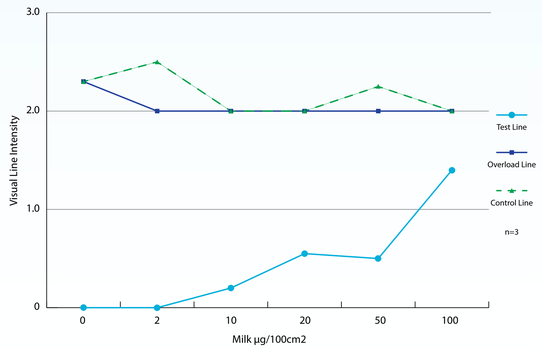
Reveal 3-D Total Milk Allergen Test was tested by nine different industry professionals for performance in CIP and juice. 18 blind samples were sent off-site for analysis, and independent testing was performed at each site. The mean recovery was 100% accurate.

| Sample | Total milk level | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Site 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % Correct | | | | | | | | |
| CIP | No detectable amount | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| CIP | 20 ppm | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| CIP | 1000ppm | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Juice | No detectable amount | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Juice | 20 ppm | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Juice | 1000 ppm | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

### SWABBING RECOVERY

Stainless steel was artificially contaminated with known levels of milk to determine swab recovery. Milk solutions equivalent to 0, 2, 10, 20, 50 and 100 µg/100 cm² in buffer were deposited evenly in 100 µL amounts in a 10 cm x 10 cm area and left to dry for 2 hours. The surface was swabbed and extracted following the test kit insert instructions. Next, 18 extracted samples were run on the device and the line intensity of the overload, test and control lines were recorded. Swabbing recovery of milk protein on stainless steel is 20 µg/100 cm².

### SURFACE SWAB TESTING




| | 0 µg/100cm² | 2 µg/100cm² | 10 µg/100cm² | 20 µg/100cm² | 50 µg/100cm² | 100 µg/100cm² |
|---|---|---|---|---|---|---|
| | % positive results | | | | | |
| Stainless steel | 0% | 0% | 33% | 100% | 100% | 100% |