ThunderBolt®/Bolt™ automation for RIDASCREEN® Total Gluten

Art. No. R7041

Verification report

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General

RIDASCREEN® Total Gluten (Art. No. R7041) is a sandwich enzyme immunoassay developed for the quantitative analysis of intact (non-hydrolyzed) gluten from gluten containing cereals (wheat, rye and barley) in oat and oat products. For detailed information about test validation, we refer to the RIDASCREEN® Total Gluten validation report.

The aim of this report is to compare the manual handling with the automated analysis. The automated analysis uses the same test kit components as the manual handling. The pipetting volumes and the incubation times are the same in both cases.

The complete verification data is collected via the ThunderBolt™. The ThunderBolt® and the Bolt™ basically use the same components (pipetting unit, photometer, shaker...) as well as the same software. Thus, only a comparative measurement is performed on the Bolt™ at the end of a ThunderBolt® verification. This measurement provides information about the identical results (curve and samples) to the ThunderBolt®. If results are comparable on both automates, a separate, complete verification study is not necessary for the Bolt™. In the case of RIDASCREEN® Total Gluten, the assay protocol can be used equally for both automates.

Test principle

The principle of the test is the antigen-antibody reaction. The wells of the microtiter strips are coated with specific antibodies against gluten proteins. By adding the standard or sample solution to the wells, gluten proteins present in the solutions will bind to the specific capture antibodies resulting in the formation of an antibody-antigen-complex. Components not bound by the antibodies are then removed in a washing step. Following the washing step, a solution containing antibody conjugated to peroxidase is added. This conjugate binds to the Ab-Ag-complex and an antibody-antigen-antibody (sandwich) complex is formed. Any unbound conjugate is then removed in another washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound conjugate converts the colorless chromogen into a blue product. A stop solution is added which results in a color change from blue to yellow. The absorbance of the solution, which is proportional to the gluten protein concentration in the sample, is measured photometrically at 450 nm.

Specificity

Information on specificity was obtained by manual pipetting only and is contained in the in the instruction for use for RIDASCREEN® Total Gluten ELISA.

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Homogenity

For testing the assay's homogeneity over the plate on the automate, one negative sample was pipetted to the microtiter strips with even numbers (nc = negative control; 48 wells in total) and one spiked gluten sample was pipetted to the microtiter strips with uneven numbers (mpr = middle positive reference sample; OD range from 0.739 - 1.037; 48 wells in total). The OD values were plotted against the corresponding microtiter strip numbers (see figure 1). Each data point represents one measurement. Evaluation clearly shows that the OD values of both samples are very consistent and a shift to higher or lower values does not appear over the plate.

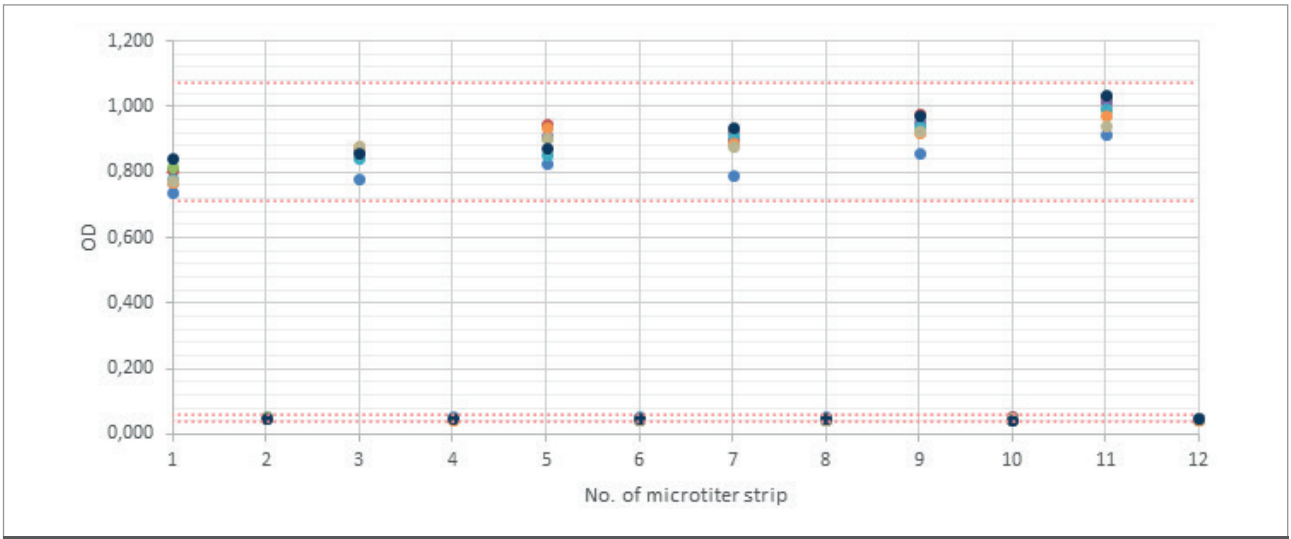

Figure 1: Plot of optical densities (OD) of a negative and a positive sample against the corresponding number of microtiter strip. Red dotted lines indicate the calculated minimum and maximum value range (mean ODs +/- 20 %) of nc and mpr.

Standard deviation and coefficient of variation of measured optical density were calculated for both samples (see table 1).

Table 1: Summary of the homogeneity test.

	mpr	nc
Mean optical density (OD)	0.892	0.048
Coefficient of variation (%)	8.2	5.1
Number of outlier	0	0

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Comparison of methods – automation and manual pipetting

Accuracy

Different samples at spike levels from 5.6 to 61.8 mg/kg gluten were analyzed with test kits from the same batch on a ThunderBolt® and by manual pipetting. Calculation of measured concentrations was performed by using the RIDASOFT® Win.NET software (evaluation for manual testing) and the ThunderBolt® software (evaluation for automated testing). Results were evaluated using the 4-parameter function. The summary, shown in table 2, reveals deviations between automatic and manual processing within an acceptable range. Matrix-related differences must be taken into account if necessary.

Table 2: Summary of the comparison of methods.

		Manual		Automation		
Sample description	Spike conc. (mg/kg)	Conc. (mg/kg)	Recovery (%)	Conc. (mg/kg)	Recovery (%)	Recovery difference automate to manual
Oats	0.00	1.10	–	0.50	–	–
Oats	5.58	5.80	103.9	4.92	88.1	15.8
Oats	10.10	7.90	78.2	8.68	85.9	7.7
Oats	17.06	15.80	92.6	17.85	104.6	12.0
Oats	33.64	32.60	96.9	33.33	99.1	2.2
Oats	61.84	53.20	86.0	58.74	95.0	9.0
Rye	20.36	23.40	114.9	27.20	133.6	18.6
Rye	14.23	12.70	89.2	14.99	105.4	16.1
Wheat	10.85	8.80	81.1	9.74	89.8	8.7
Wheat	19.75	15.50	78.5	19.25	97.5	19.0
Barley	11.15	9.20	82.5	13.26	118.9	36.4
Barley	19.95	16.10	80.7	24.68	123.7	43.0
Mean			89.5		103.8	

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Repeatability

The repeatability was determined by measuring the standard curves and one oat, wheat and barley sample respectively, with different concentrations of gluten on four successive days (Day 1, 2, 3 and 4). Every day, kit reagents were freshly prepared and a new aliquot of the sample extract was taken from the fridge. Five replicates (n = 5) were tested for each sample or standard.
The results (of the repeatability mesurment) are shown in table 3 and table 4. The repeatability study showed comparable results for standards and samples over four days with a low day to day variation in an acceptable range. Thus, the ThunderBolt® automate is able to perform the assay with reproducible results.

Table 3: Summary of the repeatability of the kit standards on four different days (Day 1 - 4). CV values were calculated from five replicates of each standard with each run.

	Standard 1		Standard 2		Standard 3		Standard 4		Standard 5		Standard 6	
	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)
Day 1	0.052	9.2	0.212	3.7	0.488	9.0	0.654	10.0	1.117	6.2	1.533	5.4
Day 2	0.058	11.4	0.167	3.5	0.258	9.1	0.467	2.6	0.838	3.4	1.272	6.9
Day 3	0.050	12.8	0.166	1.9	0.253	2.5	0.477	2.9	0.860	6.7	1.352	5.9
Day 4	0.053	12.5	0.145	5.9	0.243	3.8	0.393	4.2	0.728	11.9	1.191	9.5
Mean	0.053	11.5	0.173	3.8	0.310	6.1	0.498	4.9	0.886	7.1	1.337	6.9
SD	0.003	1.6	0.028	1.6	0.119	3.4	0.111	3.5	0.165	3.5	0.146	1.8
CV (%)	6.5		16.3		38.2		22.2		18.6		11.0	

Table 4: Summary of the repeatability of three samples (oat (o), wheat (w), barley (b)) with different concentrations of gluten on four different days (Day 1 - 4). CV values were calculated from five replicates of each sample with each run.

	Sample 1 – oat (o; 0.00 mg/kg)			Sample 2 – wheat (w; 15.00 mg/kg)			Sample 3 – barley (b; 15.00 mg/kg)		
	OD	CV (%)	Rec. (%)	OD	CV (%)	Rec. (%)	OD	CV (%)	Rec. (%)
Day 1	0.116	16.2	–	0.552	12.2	95.2	0.773	9.8	152.5
Day 2	0.094	1.9	–	0.426	3.6	117.3	0.560	8.7	161.5
Day 3	0.085	4.1	–	0.425	7.2	115.8	0.499	1.0	138.9
Day 4	0.081	2.6	–	0.345	8.2	108.5	0.420	6.7	137.7
Mean	0.094	3.7		0.437	7.8	109.2	0.563	6.6	147.7
SD	0.016	1.9		0.086	3.5	10.1	0.151	3.9	11.4
CV (%)	16.6			19.6		9.2	26.9		7.7

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On board stability

The "on board stability" was determined by measuring the standard curve and an oat, wheat and barley sample respectively with different concentrations of gluten. Reactivity was tested and repeated after leaving the components six hours inside the automate (on board). For each sample or standard, 5 replicates (n = 5) were tested. The on board stability is given for measurements after six hours, which is confirmed by variation of recoveries in a normal range. As can be seen in table 5 and 6, there is only a slight tendency to decrease over the investigated time, which is, however, still in an acceptable range.

Table 5: Summary of the on board stability of the kit standards with initial testing and testing after leaving the sample in the automate for 6 hours. CV values were calculated from five replicates of each standard with each run.

	Standard 1		Standard 2		Standard 3		Standard 4		Standard 5		Standard 6	
	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)
Day 1 - initially	0.052	9.2	0.212	3.7	0.488	9.0	0.654	10.0	1.117	6.2	1.533	5.4
Day 1 - after 6 h	0.054	7.7	0.169	2.5	0.254	9.2	0.452	5.9	0.849	3.0	1.300	4.5
Mean	0.053	8.5	0.191	3.1	0.371	9.1	0.553	8.0	0.983	4.6	1.417	5.0
SD	0.002	1.1	0.030	0.8	0.165	0.1	0.143	2.9	0.190	2.3	0.165	0.6

Table 6: Summary of the on board stability of three samples (oat (o), wheat (w), barley (b)) with different concentrations of gluten with initial testing and testing after leaving the sample in the automate for 6 hours. CV values were calculated from five replicates of each sample with each run.

	Sample 1 – oat (o; 0.00 mg/kg)			Sample 2 – wheat (w; 15.00 mg/kg)			Sample 3 – barley (b; 15.00 mg/kg)		
	OD	CV (%)	Rec. (%)	OD	CV (%)	Rec. (%)	OD	CV (%)	Rec. (%)
Day 1 - initially	0.116	6.2	–	0.552	12.2	95.2	0.773	9.8	152.5
Day 1 - after 6 h	0.091	2.5	–	0.412	7.7	120.3	0.490	4.9	143.9
Mean	0.104	4.4		0.482	10.0	107.8	0.632	7.4	148.2
SD	0.018	2.6		0.099	3.2	17.7	0.200	3.5	6.1

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Carry-over testing

In automates using only one needle for pipetting a carry-over effect may occur. This means that high positive samples may contaminate following negative samples due to insufficient rinsing of the needle. This is why the R-Biopharm 'Field Service Unit' analyses negative samples following high positive samples directly for each assay to demonstrate the reliable and robust rinsing of the needle. More precisely, two wells of a high positive sample, starting with 8,000 mg/kg, are followed by four wells of blank. Using this scheme, always two wells with decreasing concentrations of 4,000 mg/kg, 2,000 mg/kg, 1,000 mg/kg, and 500 mg/kg are followed by four wells of blank.

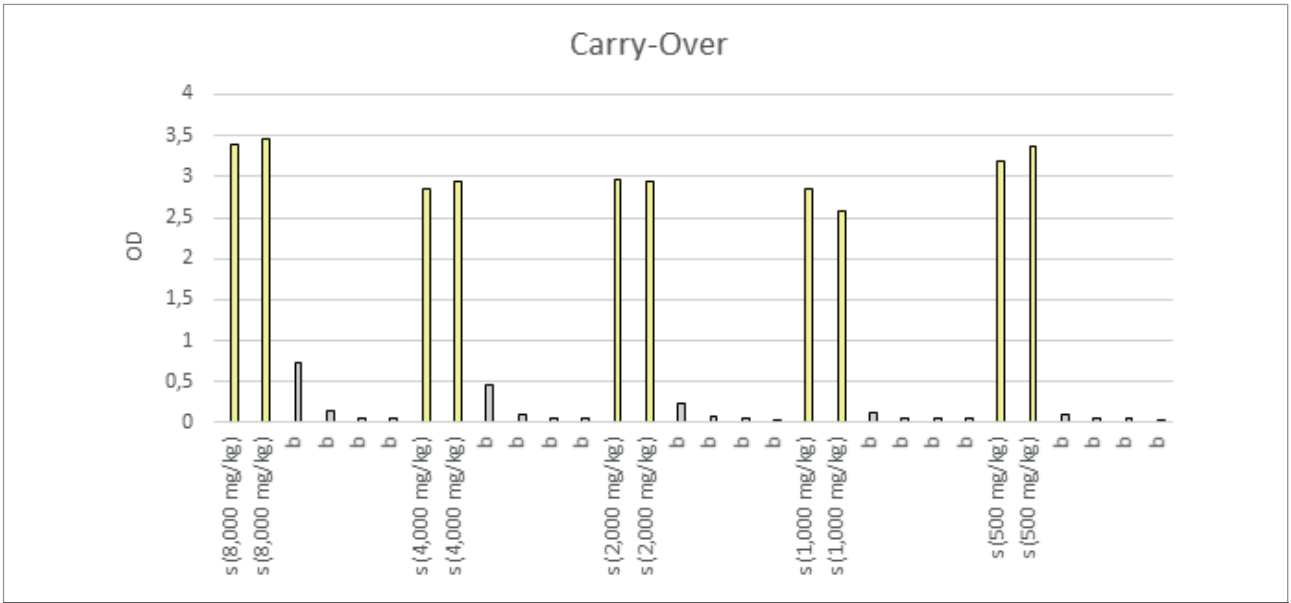

Figure 2: Results of the carry-over testing. Spiked (s) and blank (b) samples were tested in an alternating scheme.

The samples were prepared by spiking the dilution buffer from the test kit to the corresponding concentrations. For the blank samples, the pure dilution buffer was used.

In figure 2, the OD values of the spiked and blank samples are shown. All highly positive samples result in a high OD (> standard 6), whereas the blank samples have expected low OD values. For high positive samples up to a concentration of 1,000 mg/kg, no carry-over effect was observed to blank sample wells. At even higher concentrations, slightly increased values were measured only in the first well of four consecutive blank well samples. At the latest, in the second well this effect was no longer visible, because the washing steps between these wells was sufficient.

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Conclusion

The results prove that the ThunderBolt® automate can be used to analyze gluten samples in the RIDASCREEN® Total Gluten test. The determined recovery is comparable to the result of the manual testing. Repeatability with reproducible results is given from run to run, that there is only a slight tendency to decrease over the investigated time, which is, however, still in an acceptable range.
The carry-over testing demonstrated that the pipetting needle is sufficiently cleaned during the processing in the automate. Carry-over contamination from samples with less than 1,000 mg/kg to blank samples in the following well can be excluded. Only highly contaminated samples may cause a slight carry-over into the next well. However, a possible carry-over effect will be detected immediately when working in duplicate.

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