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validation report for
TaqMan® Roundup Ready Quantification Kit

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2. Purpose

The aim of the present document is to validate the method for determining the percentage of transgenic Roundup Ready soy (MON-Ø4Ø32-6;GTS 40-3-2) with respect to the total soy present in a sample using the TaqMan® Roundup Ready Quantification kit. This soy accounts for approximately 80% of all soy cultivated worldwide. It is the most widespread transgenic event and that which generates the most problems.

3. References

Guideline UNE-EN ISO/IEC 17025:2005
Guideline UNE-EN ISO/IEC 21570:2005
Verification of analytical methods for GMO testing when implementing interlaboratory validated methods.
Guidance document from the European Network of GMO laboratories (ENGL)

4. Definitions

Validation: Confirmation by examination and the provision of objective evidence to demonstrate compliance with certain requirements for the expected specific use [source: ISO17025]

Precision: The degree of concordance between the results of independently obtained measurements under the conditions established.

Repeatability: Precision under conditions in which the results of a measurement are obtained using the same method, by the same operative, and using the same measurement instrument.

Reproducibility: Precision under conditions in which the results of a measurement are obtained using the same method and same measurand, but with different operatives and different measurement instruments.

Specificity: The degree to which a method can determine a particular analyte in a complex mixture without interference from other components in the mixture.

Limit of detection: The lowest value of an analyte in a sample that can be examined, that can be detected but not necessarily quantified exactly. Absolute limit of quantification (LOQabs): The lowest quantity or concentration of a target DNA that can be reliably quantified with an acceptable level of precision and trueness. This limit is expressed as a DNA copies.

Relative limit of quantification (LOQrel): The lowest amount of analyte in a sample which can be reliably quantified with an acceptable level of precision and trueness. This limit is expressed as a percentage.

5. Introduction

The TaqMan® Roundup Ready Quantification Kit allows the percentage of Roundup Ready soy with respect to total soy present in a sample to be determined. Each sample analysis includes two real-time PCR steps. One of these allows the total amount of soy present in the sample to be quantified, whereas the other allows the amount of Roundup Ready soy in the sample to be determined.

The kit includes a standard consisting of plasmid DNA containing a copy of each of the targets used during the analysis. Comparison of the results obtained for samples with this standard allows a relative quantification to be performed, thus providing the percentage of Roundup Ready soy with respect to total soy present in each sample.

6. Validation Assay

6.1. Specificity

As well as the theoretical specificity analyses performed when designing the oligonucleotides and probes used in the kit, specificity assays have been performed using various transgenic varieties. The acceptance criterion used for the specificity parameter is that the PCR system developed should only produce amplification for the event for which the amplicon is expected to be detected, in other words for Roundup Ready soy.

A table listing the varieties used during the assay is provided below (Table 1). Varieties containing different percentages of transgenic material were used during this assay.

Table 1. Varieties used during the specificity assay for TaqMan® Roundup Ready Quantification Kit

Standard transgenic varieties				
Maize Soy	Soy	Oilseed Rape	Cotton	Sugarbeet
GA21	RR	T45	MON1445	H7-1
MON810	A2704-12	GT63	MON531	
MON863	MON89788			
BT176				
BT11				
NK603				
TC1507				
T25				

6.2. Limit of quantification (LOQ) and limit of detection (LOD)

To establish the limit of detection (LOD) for the TaqMan® Roundup Ready Quantification Kit, real-time PCR assays were performed using 21 replicates of samples containing different DNA copy numbers. The number of copies assayed was: 5, 2.5 and 1 copy of the kit's standard for both soy and Roundup Ready soy.

Table 3. Results for the limit of detection assay for the TaqMan® Roundup Ready Quantification Kit.

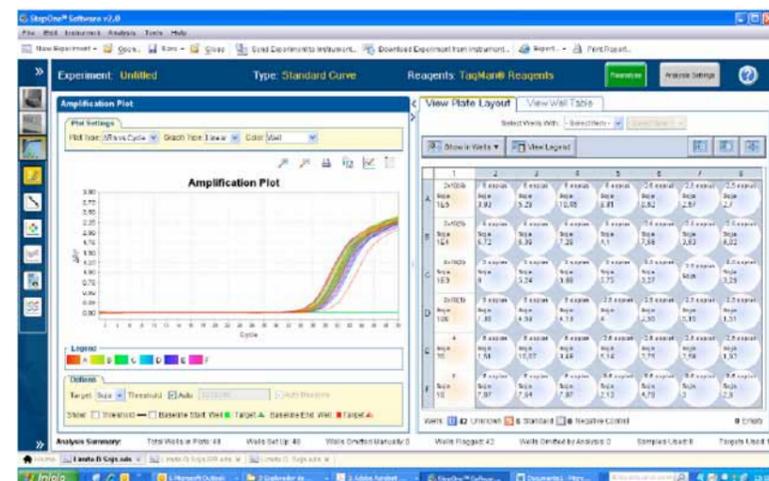
Results for limit of detection (LOD)			
	5 copies	2.5 copies	1 copies
Soy	100% [21/21]	>95% [20/21]	>85% [18/21]
Roundup Ready Soy	100% [21/21]	100% [21/21]	>76% [16/21]

On the basis of the results obtained, the limit of detection for the kit can be established as 2.5 DNA copies. The following graphs show the limit of detection for 5 and 2.5 DNA copies for each determination using the kit.

Chart 1. LOD for RR soy



Chart 2. LOD for soy



The **absolute limit of quantification (LOQabs)** for the TaqMan® Roundup Ready Quantification Kit has been determined using the standard provided with the kit, which consists of a plasmid containing targets for the endogenous soy gene, known as lectin, and for the transgenic event GTS-40-3-2 present in Roundup Ready soy.

To perform the validation, real-time PCR assays were performed on 21 standard replicates containing 20 or 10 DNA copies for both soy and Roundup Ready soy. As can be seen in the following table, the absolute limit of quantification has been established as 20 copies, which is the value for the last point on the standard curve. Any sample for which a lower value is obtained should be considered to be extrapolated and therefore the result cannot be expressed quantitatively.

Table 1. Results for the limit of quantification assay for the TaqMan® Roundup Ready Quantification Kit

Results for limit of quantification (LO Qabs)					
	No. DNA copies	No. replicates	Mean	Deviation	CV
Soy	10	100% [21 / 21]	1.43	4.36	38%
	20	100% [21 / 21]	17.84	4.28	24%
Roundup Ready Soy	10	100% [21 / 21]	13.98	3.49	25%
	20	100% [21 / 21]	21.38	2.49	12%

The acceptance criterion indicates that the limit of quantification should have a CV<25%. It has been confirmed that the CV is less than 25% in all studies performed, even in the ranges with a copy number of 20. The following graph are an examples of the limit of quantification results obtained for 20 DNA copies in each determination using the kit.

Chart 3. LOQabs for soy



Chart 4. LOQabs for soy



The **relative limit of quantification (LOQrel)** for the TaqMan® Roundup Ready Quantification Kit has been determined using a standard DNA that contains 0,01% of Roundup Ready soy.

To perform the validation, real-time PCR assays were performed on 11 standard replicates of this standard DNA which contains 0,01% of Roundup Ready soy. The acceptance criterion indicates that the relative limit of quantification should have a CV ≤ 25%

Table 2. Results for the relative limit of quantification assay for the TaqMan® Roundup Ready Quantification Kit

Results for relative limit of quantification (LOQ)				
Event analysed	Standard used	Mean	Deviation	CV
Soja RR / Soja	RR 0.01%	0.011	0.002	23.6%

6.3. Accuracy and Precision

Multiple assays under the conditions of repeatability and reproducibility, using certified standards for Roundup Ready soy (IRMM) with values of 10%, 1% and 0.1%, were performed to determine the accuracy and precision. These standards were also used with different DNA concentrations per reaction. The statistical parameters obtained during the validation are summarised in the following table:

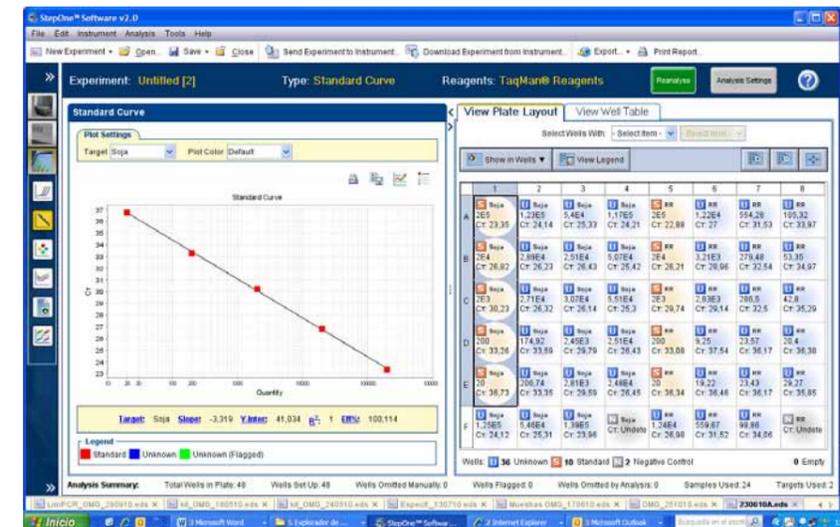
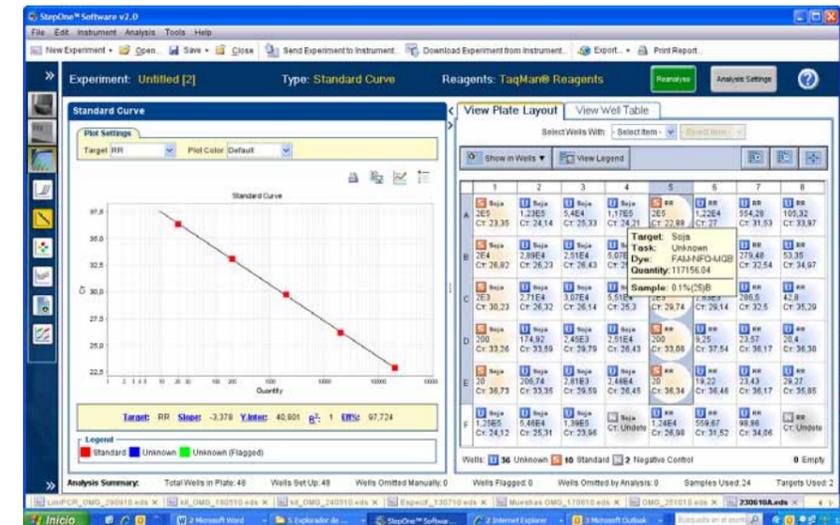
Table 4. Results for the accuracy and precision assays.

Results for Accuracy and Precision							
Event Analysed	Standard used	Operative 1			Operative 2		
		Mean	Deviation	CV	Mean	Deviation	CV
RR Soy / Soy	10% RR	9.98	1.36	13.7%	10.24	1.40	13.6%
	1% RR	0.99	0.23	23.0%	1.04	0.23	21.7%
	0.1% RR	0.09	0.02	22.1%	0.11	0.03	23.7%

The results obtained from the efficiency amplification and from R2 coefficient for soy and RR meet the acceptance criteria. The average value of the slope for the standard curve shall be in the range of $-3.6 \leq \text{slope} \leq -3.1$, and the average value of R2 shall be ≥ 0.98

RR Soy			Soy		
Slope	R ²	Efficiency	Slope	R ²	Efficiency
-3,392	0,999	97,3%	-3,378	0,999	97,9%

Chart 5: Efficiency and R² for RR soy and soy



7. Results

Three results are possible for each amplification reaction of both soy and Roundup Ready soy:

- **Not detected:** No amplification in the sample. The amplification curve is flat.
- **Not quantifiable:** Amplification is detected in the sample but to an extent lower than the last point on the curve. When the Ct for the sample is greater than the Ct for the 20-copy standard, it can be concluded that the analyte is present in the sample but is not quantifiable.
- **Quantifiable:** Amplification is detected in the sample to an extent greater than the last point on the curve. When the amplification Ct for the sample is interpolated between the values for the standard points, the quantitative result can be considered to be reliable and can be used to calculate the percentage of Roundup Ready soy.

The following formula should be used to calculate the percentage of Roundup Ready soy with respect to total soy present in the sample:

$$\% \text{ RR Soy} = \frac{\text{No. of copies of RR soy} \times 100}{\text{No. of soy copies}}$$

8. Conclusion

All the results obtained in this validation report allow us to assess the suitability of the TaqMan® Roundup Ready Quantification Kit to quantify the Roundup Ready soy in DNA obtained from any food or feed, with the necessary precision and efficiency.