Analysis report ORAC Europe BV

		initials investigator
Customer name:	**Hak Agrofeed BV** ***att.* Mr. B. Hak** **Leemansstraat 2** **4251 LD Werkendam** **The Netherlands**	
Amount of samples delivered:	10 samples	**EW**
Date of sample arrival:	Wednesday, January 6, 2010	**EW**
Sample condition at arrival:	***OK*** [X] ***Damaged*** [ ] ***Other*** [ ] *(see remarks)*	
Sample storage conditions:	Upon arrival, samples were stored at 4ºC, in the dark.	**EW**
Remarks:	Samples were presented as individually wrapped tomatoes. Tomatoes were divided into two separately labeled groups.	**EW**

Customer's description of samples:

The delivered tomatoes were divided into two groups:
a control group (black label, n=5) and an *Immutines*-treated group (1 mL/m^2/week, orange/brown label, n=5).
The control group consisted of untreated tomatoes. Control tomatoes were labeled: C-1, C-2, C-3, C-4 and C-5.
Immutines-treated tomatoes (1 mL/m^2/week) were labeled: I-1, I-2, I-3, I-4 and I-5.

All tomatoes were grown at the nursery of A.W. Vahl in IJsselmuiden, The Netherlands.

Information about *Immutines* is provided in the appendix of the ORAC Europe report of September 23, 2008.

ORAC Europe sample preparation:

Of each tomato within the two groups, the individual weight was determined (*see results section*).
All five tomatoes of each group (control vs. *Immutines*-treated) were thoroughly grinded using a laboratory grinder. From each of the two resulting mixtures, 5 gram was accurately weighed in labeled glass test-tubes.
To extract the hydrophilic contents from each sample, 20 mL of an acetone/water/acetic acid solution (140:59:1, v/v) was added to each test-tube. This solution (abbreviated as AWA) is commonly used to extract hydrophilic constituents from food samples.
All sealed test-tubes with samples were placed in an ultrasonication bath for 15 min. Hereafter, all samples were thoroughly vortexed for 1 min, and placed back in the ultrasonication bath for another 15 min.
All tubes were vortexed again for another minute and finally, all tubes were centrifuged at 800 x g for 15 min.
Supernatants were carefully collected and stored in dark glass bottles at 4°C until further use in the hydrophilic ORAC assay.

		initials investigator
Date of sample preparation:	Thursday, January 7, 2010	EW
Required assay:	**Hydrophilic ORAC assay**	EW
Date(s) of testing:	Friday, January 8, 2010	EW
Responsible investigator:	Dr. E. van den Worm 	EW
Report finalization date:	Monday, January 11, 2010	EW
Remarks:	*- no remarks -*	EW

Short description of performed assay:

Samples provided by Hak Agrofeed BV were tested for their ***O**xygen **R**adical **A**bsorbance **C**apacity* (ORAC), using the commonly accepted and well-validated hydrophilic ORAC assay with fluorescein as fluorescent probe and with AAPH (2,2'-azobis (2-methyl-propionamidine) dihydrochloride) as a physiological relevant peroxyl radical generator. Kinetic fluorescence profiles were detected using an automated fluorescence reader (*Thermo Fluoroskan Ascent*). Fluorescence was monitored every minute for 1 hr. at 37ºC, using an excitation wavelength of 485 nm and an emission wavelength of 538 nm. *(Assay performed in accordance to: Prior et al., J. Agric. Food. Chem. 53(10): 4290-4302; 2005.)*

In the ORAC assay, Trolox (a water-soluble derivative of vitamin E) is used as an internal standard. Therefore, the results of the ORAC assay are expressed as µmol Trolox equivalents (TE) ***per 100 g of test-sample***. This is a standard way of expressing ORAC values.

Each sample was diluted in freshly prepared 75mM sodium phosphate buffer (pH = 7.4) shortly before the experiment.

All reagents were freshly prepared prior to the experiment. All solutions were kept in the dark at 37ºC except the AAPH solution which was kept on ice (in the dark) until use.

From the obtained experimental data, final ORAC values were calculated using the 'area under the curve' (AUC).
The net AUC was obtained by subtracting the AUC of the blank from that of the sample. The relative ORAC value (expressed as Trolox equivalents) was calculated by extrapolation from the Trolox calibration curve (AUC_{Trolox} vs. [Trolox]).
ORAC values are expressed as mean values ± Standard Deviation (S.D.).

Remarks:

Before all dilution steps and final addition to test-plate, all sample dilutions were carefully vortexed.

TEST RESULTS:

Tomato nr.	Weight (gram)
C-1 (Control)	129.92
C-2 (Control)	149.64
C-3 (Control)	127.57
C-4 (Control)	135.89
C-5 (Control)	132.26
I-1 (*Immutines*, 1 mL/m^2/week)	127.96
I-2 (*Immutines*, 1 mL/m^2/week)	136.70
I-3 (*Immutines*, 1 mL/m^2/week)	169.51
I-4 (*Immutines*, 1 mL/m^2/week)	177.92
I-5 (*Immutines*, 1 mL/m^2/week)	275.02

Samples	ORAC value (µmol TE / 100 g.)*
Control tomatoes (C)	**295 ± 9.2 (mean ± S.D.)**
***Immutines*-treated tomatoes (1 mL/m^2/week, I)**	**335 ± 7.1 (mean ± S.D.)**

***** *As stated previously, ORAC values are expressed as µmol TE per 100 g of test sample. If required, customer can extrapolate these ORAC values to µmol TE per tomato or µmol TE per serving.*

NB: 1 µmol Trolox Equivalents (TE) equals 250 µg Trolox

	Antioxidant capacity (% of control)	Increase in antioxidant capacity (relative to control)
Control tomatoes (C)	**100**	**-**
***Immutines*-treated tomatoes (1 mL/m^2/week, I)**	**113.6 ± 2.5**	**13.6 %**

Responsible Investigator:

Dr. E. van den Worm
(*CEO*, ORAC Europe BV)

……

• **ORAC Europe BV** • PO BOX 80082 • 3508 TB Utrecht • The Netherlands •
• Rabobank: 12 77 66 456, Utrecht • IBAN: NL41RABO 0127 7664 56 •
• BIC: RABONL2u • KvK 30222065 • VAT nr. NL8174.77.615.B01 •
• Tel: 00 31(0)302535933 • Fax:00 31(0) 302536941 •
• e-mail: info@orac-europe.com •
• www.orac-europe.com •