Towards
Reference Materials for Food Allergen and Gluten-Free Analysis

Roland Poms

MoniQA Association, Vienna, Austria
www.moniqa.org

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SIXTH FRAMEWORK PROGRAMME

MoniQA Association

MoniQA Global Food Safety Network

…providing expertise and services for effective food safety management..

…for food manufacturers, food retailers, international trade, food analytical laboratories, food control and regulatory bodies

- Non profit-distributing association
- Established in July 2011
- Membership based
- Secretariat at ICC HQ in Vienna, Austria

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Research | Industry | Consumers | Policy Makers

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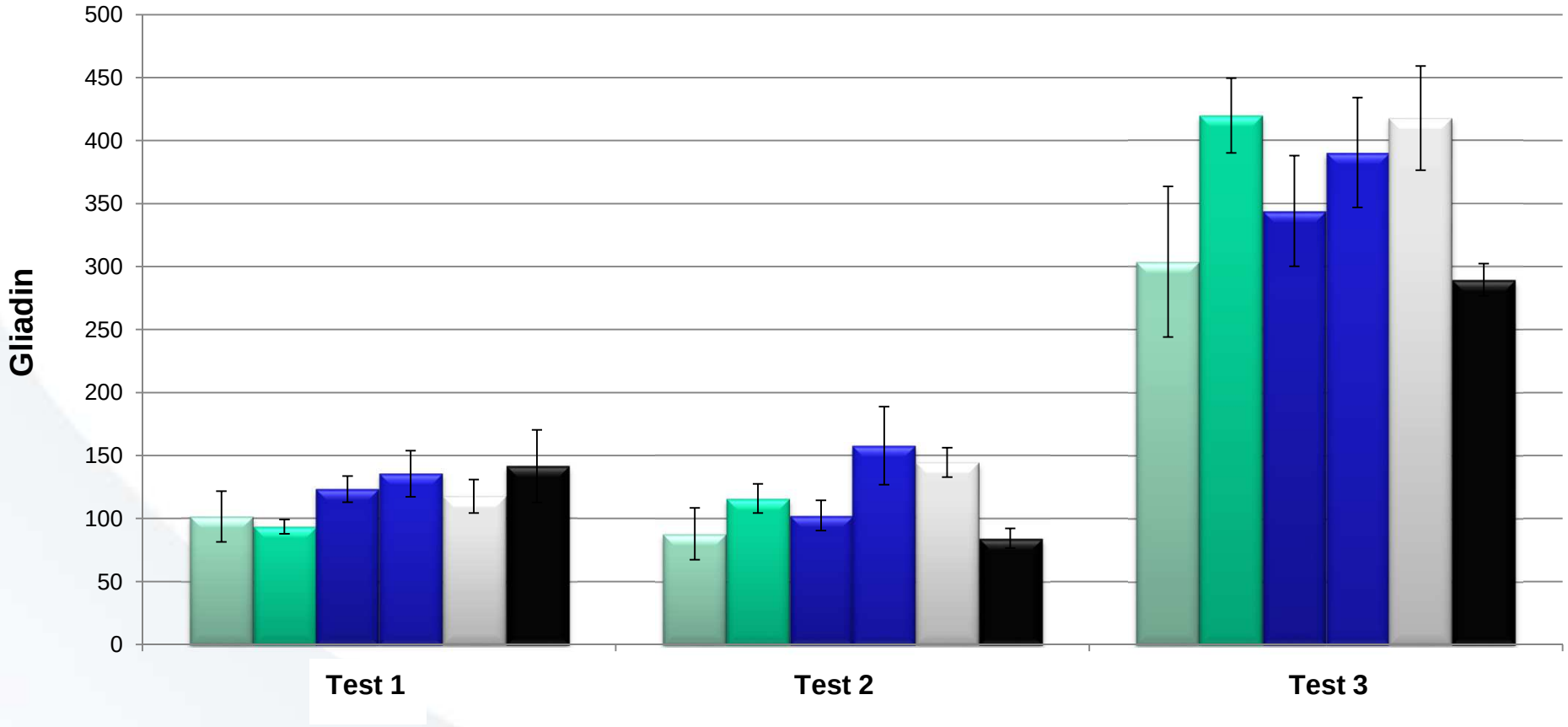
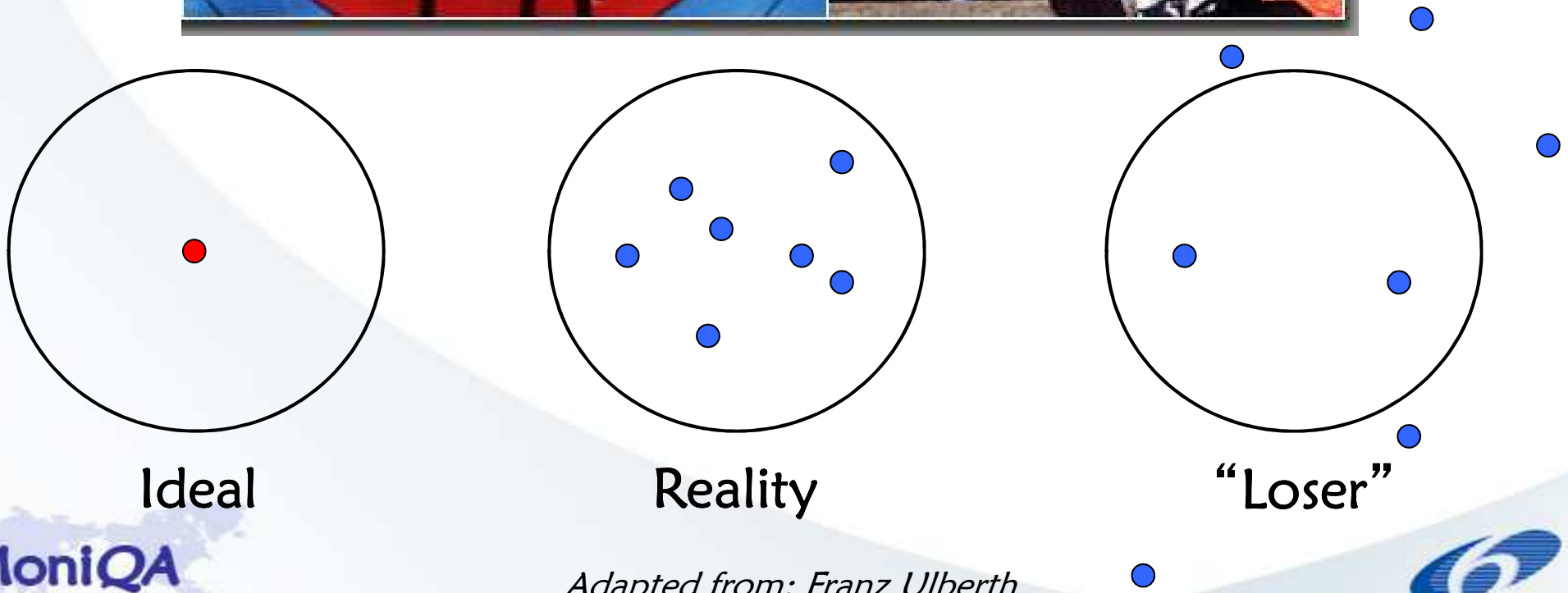
Example: Gliadin analysis by 3 different tests



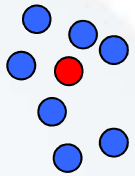
How can I know
that
my analysis is correct?

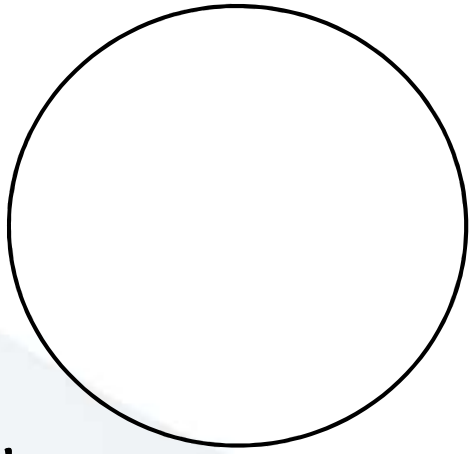
Uncertainty – reliability of analytical results



Adapted from: Franz Ulberth, Measurement Uncertainty Workshop, Budapest, March 9, 2008

Trueness – reliability of analytical results



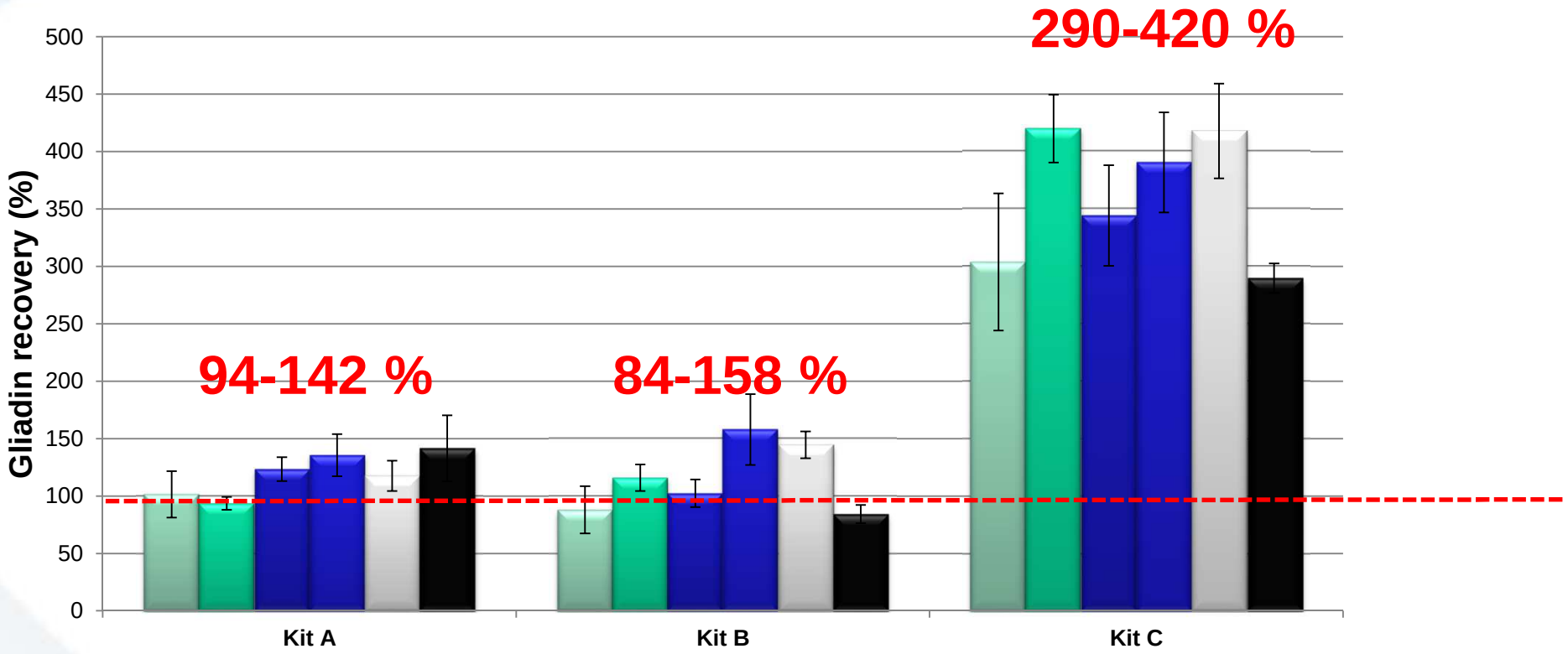


…looking good

…but way off

Adapted from: Franz Ulberth,
Measurement Uncertainty Workshop, Budapest, March 9, 2008

Using a Reference Material



- Flour from one Hungarian wheat variety (MV-Magvas; Harvest year: 2011)
- Flour from one Hungarian wheat variety (MV-Magvas; Harvest year: 2012)
- Blended flour 1 (Mv-Magvas, MV-Mazurka, MV-Verbunkos, Dekan, Yumai-34; Harvest year: 2011)
- Blended flour 1 (Mv-Magvas, MV-Mazurka, MV-Verbunkos, Dekan, Yumai-34; Harvest year: 2012)
- Blended flour 2 (MV-Magvas, Bezostaja-1, Glenlea, Hereward, Soissons; Harvest year: 2012)
- PWG gliadin

Hajas, L. , Tömösközi, S. , publication in preparation

MoniQA Working Group

Food Allergen Reference Materials

Coordinator: Roland Poms (MoniQA, Austria)

WG Committee: Jupiter Yeung (**Nestle**, USA), Terry Koerner (**Health Canada**, Canada), Thomas Holzhauser (**Paul Ehrlich Institute**, Germany), Peter Koehler (**Prolamin WG**, Germany), Sigrid Haas-Lauterbach (**R-Biopharm**, Germany), Bert Popping (**AOAC**, USA), Joe Baumert (**FARRP**, USA), Robin Sherlock (**FACTA/Allergen Bureau**, Australia), Franz Ulberth (**European Commission, IRMM**, Belgium), Eric Garber (**FDA**, USA),…hosted by Kurt Johnson (**Trilogy Laboratories**, USA).

Contributors and stakeholders: Research institutes, test kit providers, method developers, food businesses, policy support, a.o.

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Priority Allergen Testing Materials

- **Basic materials (minimally processed), sufficiently characterised, as basis to produce incurred materials and extracts at different concentrations, model foods (e.g. rice cookie)**

- Gluten/Gliadin, basic materials: flours of wheat, rye, barley, oat, rice

- Milk: dried skim milk powder

- Egg: dried full egg powder, dried egg white powder

- Hazelnut: flour

- Peanut: defatted peanut flour

Characterization and Quality Control

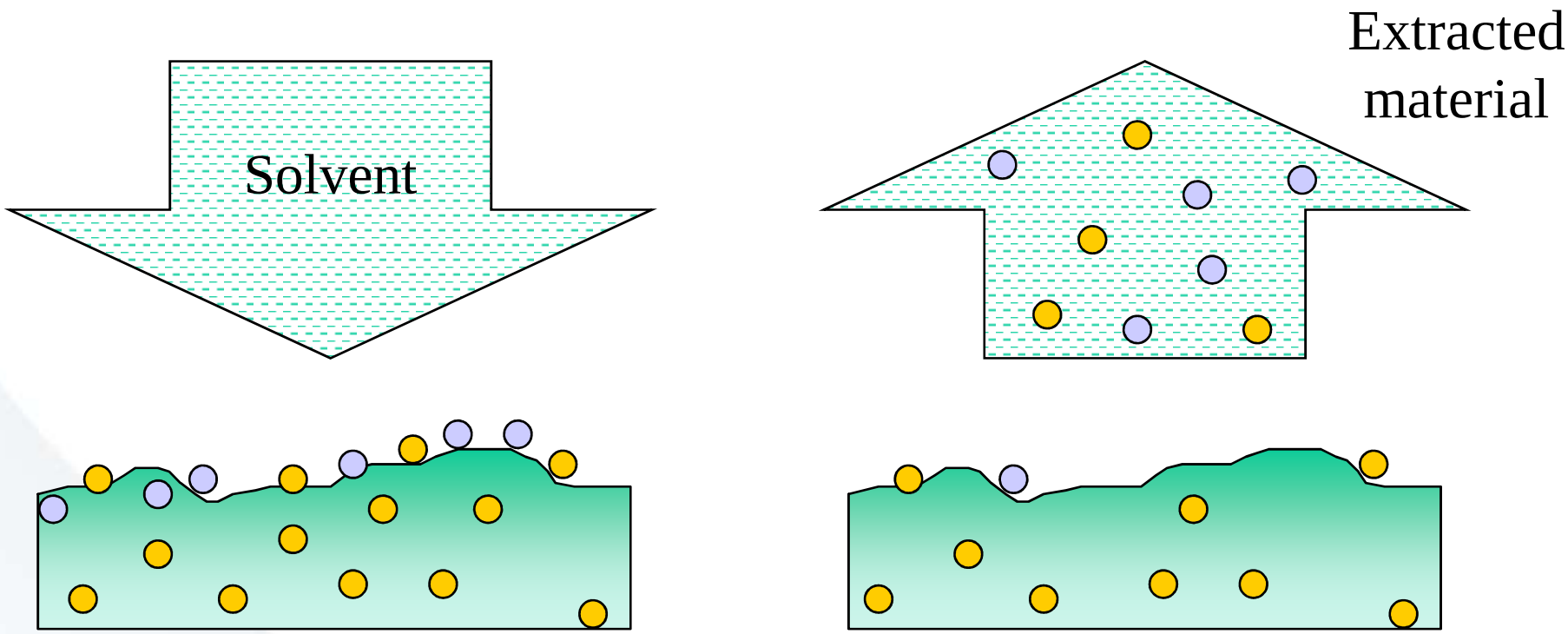
Allergenic food Commodities

Traceability documentation, provider's data
Milling, sieving, packaging, particle size (40 µm)
Protein, fat, moisture, ash analysis
Protein profile by 2D electrophoresis
Mass spec peptide profile (as available)
Storage (4 ° C, -20 ° C, -80 ° C), stability testing

Incurred RMs

Production parameters documented
Milling, sieving, packaging, particle size (40 µm)
Protein, fat, moisture, ash contents
Specific analysis by ELISA, PCR, LC-MS…
Homogeneity (particle size, allergen analysis,
Validated concentration (ring trial)
Minimum sample intake
Mass spec peptide profile (as available)
Storage (4 ° C, -20 ° C, -80 ° C), stability testing
Shelf life

Incurred vs spiked controls



Adapted from Steven Ellison, Joint IAM/MoniQA Workshop for Codex Alimentarius Delegates, Budapest 2010

MoniQA QC Material for
Milk Allergen Analysis

- Skim Milk Powder was sourced September 2014 after the MoniQA Task Force Meeting at the last AOAC Annual Meeting held in Florida.
- Production of materials was outsourced to Trilogy Analytical Laboratory, Washington, Missouri, USA
- Stored at Trilogy at -16° C +/- 1° C
- The material has analytical data for protein, fiber, moisture, carbohydrate and ash as well as a protein profile analysis.

Certificate of Analysis
issued by Trilogy Analytical Laboratory

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Reference Material
for the
Detection of Milk Proteins
Based on Skim Milk Powder
Product Code: SMP
Art. No.: MQA 092014

Certificate of Analysis

Analyte	Lab 1	Lab 2	Lab 3	Average *	1 SD	2 SD range
	Method Utilized for Determination				(grams)	(grams)
Ash	AOAC 923.03	AOAC 942.05	AOAC 930.30	7.56 g/100 g	0.15 g	7.26 – 7.86
Carbohydrates	Calculated	Calculated	Calculated	51.61 g / 100 g	0.52 g	49.97 – 52.65
Fat	AOAC 989.05 Base Hydrolysis	AOAC 954.02 Acid Hydrolysis	AOAC 989.05 Mojonnier	< 1.0 g/ 100 g		
Moisture	AOAC 925.09	AOAC 925.09	SMEDP 15.111	5.01 g / 100 g	0.40 g	4.21 – 5.81
Protein (N x 6.38)	Kjeldahl AOAC 979.09	Kjeltec AOAC 2001.11	Kjeldahl AOAC 991.20.1	35.39 g / 100 g	0.20 g	34.99 – 35.79

*this value represents the average of three independent external laboratory determinations. Each independent laboratory provided a single determination utilizing the methodology listed. The averaged result and 1 SD value are reported as well as the 2 SD range.

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Gel Electrophoreseis Protein Identification

Protein Identification	Band	Average	± SD
Unknown	1	2.2	0.14
Unknown	2	0.60	0.09
Unknown	3	1.5	0.13
Unknown	4	0.76	0.12
Lactoferrin	5	1.3	0.04
Unknown	6	0.89	0.04
Bovine Serum Albumin	7	1.1	0.03
IgG Heavy Chain	8	2.5	0.05
Unknown	9	1.9	0.05
Alpha-casein	10	31.7	0.18
Beta-casein, K-casein	11	32.0	0.39
Para-K-casein	12	1.1	0.06
Unknown	13	1.5	0.18
Beta Lactoglobulin	14	14.3	0.09
Unknown	15	0.56	0.05
Alpha-lactalbumin	16	4.6	0.18
Unknown	17	1.0	0.06

Method: SDS Slab Gel Electrophoresis by method of Laemmli, (UK Nature 227: 680-685, 1970) as described by Burgess-Cassler et. al. (Clin. Chem. 35:2297-2304, 1989; second dimension) using a 12% acrylamide slab gel (125 mm length x 150 mm width x 0.75 mm thickness) overlaid with a 25 mm stacking gel. Electrophoresis was performed using a 15 mAmp/gel for about 3.5 hours at which time bromophenol blue front had migrated to the end of the slab gel. The gel was stained with Coomassie blue, destaine in 10% acetic acid until a clear background was obtained, then dried between cellophane sheets. The stained gel was digitized over the appropriate optical density range using a calibrated GE Healthcare Image Scanner III. Stain density in individual protein bands as a percentage of stain per line were quantified using Phoretix software.

Certification Date: September 2014

Expiry date: Sept 2019

Signed: Carrie Maune

Carrie Maune for Trilogy Analytical Laboratory

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Samples available for further testing

- Packets contain an overfill of 5 grams and are contained in a zippered foil pouch
- Stored at -16° C
- Planned contingent to last for about 5 years with 7000 packets per commodity
- About 1000 packets ready
- About 20 laboratories have signed up for testing by ELISA and LC-MS, other?

MoniQA QC Material
Blank for Spiking Experiments and Basis of Incurred

- Extensive sourcing was done to secure rice flour, potato starch, xanthan gum, baking powder and baking soda with allergen levels below detection for Total Milk, Gluten, Soy, Egg, peanut and Hazelnut.
- Material was pre-sampled and tested in various laboratories to confirm allergen results.
- Large amounts of the tested “clean” lots were ordered and repackaged in large foil zippered packages.
- All products are stored at Trilogy under -16° C conditions.

MoniQA QC Material

Rice Cookie Incurred with Milk

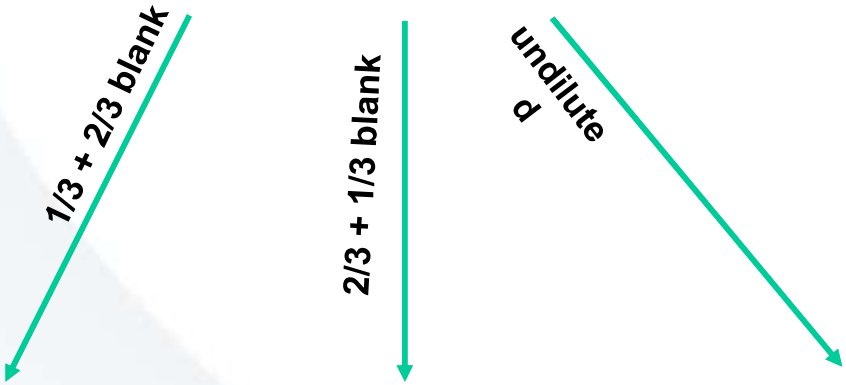
1) Dry Skim Milk was weighed, dissolved in water and incorporated in cookie dough mixtures at starting concentrations of **100 ppm and 500 ppm**, respectively
2) **Baking at 165° C for 20 min**
3) **Grinding and sieving at mesh 35/40**
4) Production of lower concentrations by blending blank and incurred material to desired content: **0, 5, 10, 50 ppm SMP** corresponding to approximately **0, 1.8, 3.5, 16.8 mg SMP protein/kg incurred cookie**.
5) Packaging and storage
6) Testing for homogeneity and recovery (by ELISA) at various stages
7) Inter-laboratory testing by ELISA and MassSpec

MoniQA QC Material

Rice Cookie Incurred with Milk

100 ppm SMP

98.6 ppm SMP
34.89 ppm milk protein



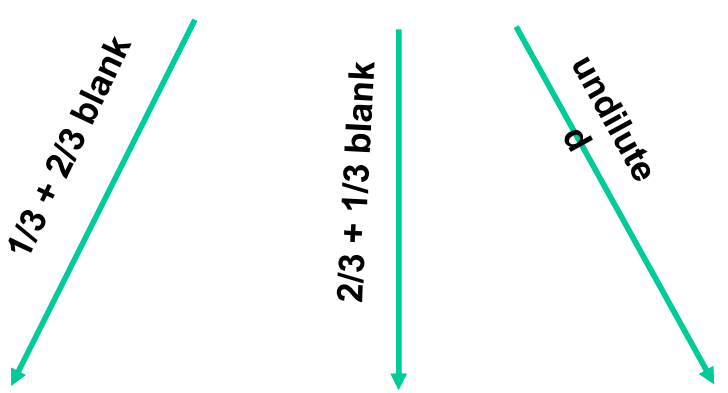
11.63 ppm milk protein **Low A1**

23.26 ppm milk protein **Low A2**

34.89 ppm milk protein **Low A3**

500 ppm SMP

498.3 ppm SMP
176.35 ppm milk protein



58.78 ppm milk protein **High A1**

117.56 ppm milk protein **High A2**

176.35 ppm milk protein **High A3**

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Preliminary Batch Results – “Total Milk”

BASIC HOMOGENEITY

	ELISA #1	ELISA #2
Low A1	7.6 %	1.6 %
Low A2	12.7 %	12.8%
Low A3	8.0 %	7.5%
High A1	5.3 %	8.7%
High A2	Outlier	4.2%
High A3	4.6 %	12.7%
Average	**7.6 %**	**7.9 %**

BASIC RECOVERY

	ELISA #1	ELISA #2
Low A1	92.0 %	62.5 %
Low A2	75.2 %	76.1 %
Low A3	65.6 %	73.3 %
High A1	83.4%	71.1 %
High A2	55.4 %	70.5 %
High A3	63.7 %	62.2 %
Average	**72.6 %**	**69.3 %**

Gluten/Gliadin RM

1. **Raw materials:** Wheat, rye, barley and oat, rice, corn
2. **Format:** Commodity flour
3. **Incurred materials:** different concentrations of wheat/rye/barley flour in rice cookie
4. **Selection of variety:**

   - setting criteria

   - quantitative

   - qualitative

Considerations

→ Reference Material (RM): as representative as possible for the multitude of wheat cultivars grown around the world

? Importance of geographic origin and other variables: cultivar, climate, year of harvest

→ Selection of a representative cultivar

? White flour or whole wheat flour

→ White flour (better stability)

? One cultivar or a mixture of several cultivars

→ One cultivar (problems with a mixture are homogeneity and high complexity)

? Isolation of gluten fractions (similar to PWG-gliadin)

→ Possible in cooperation with the Prolamin Working Group

RM Development for GLU/GLI analysis

Collection of seeds	Production of white flour	Characteri-sation	Production of flour	Incurred material
35 wheat varieties (Australian, Austrian, Canadian, Chinese, German, Hungarian, etc.) **2 Rye varieties 4 Barley varieties**		**NIR Spectrum** Infratec Grain Analyzer 1241 (850-1048 nm) • NIRSystems 6500 (400-1098, 1100-2498 nm) **Wet and dry gluten** (Glutomatic System) **1D SDS PAGE RP- and SE-HPLC Western Blotting**		**ELISA** **2D SDS PAGE**

Criteria for selection of variety?

Median Glia/Glu rate?
Median Protein/Gluten content?
Mostly used variety

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DFA Lebensmittelchemie

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Selection criteria

Based on means of all measurements (35 wheat flours/mixtures) ± standard deviation

Quantitative Criteria

Crude protein content (Dumas method):	11.8 ± 1.5 %	(10.3 - 13.3 %)
Ratio prolamin/glutelin:	2.6 ± 0.5	(2.1 - 3.1)
Ratio LMW/HMW:	2.1 ± 0.6	(1.5 - 2.7)
Content of ω5-gliadin:	3.9 ± 1.7 %	(2.2 - 5.6 %)
Content of ω1,2-gliadin:	6.5 ± 2.4 %	(4.1 - 8.9 %)
Ratio α-gliadin/γ-gliadin:	1.4 ± 0.2	(1.2 - 1.6)

Qualitative Criteria

HMW-GS:	at least 5 HMW-GS
γ-gliadin:	no pronounced peaks after an elution time > 19.5 min

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DFA Leibniz Institut Deutsche Forschungsanstalt für Lebensmittelchemie

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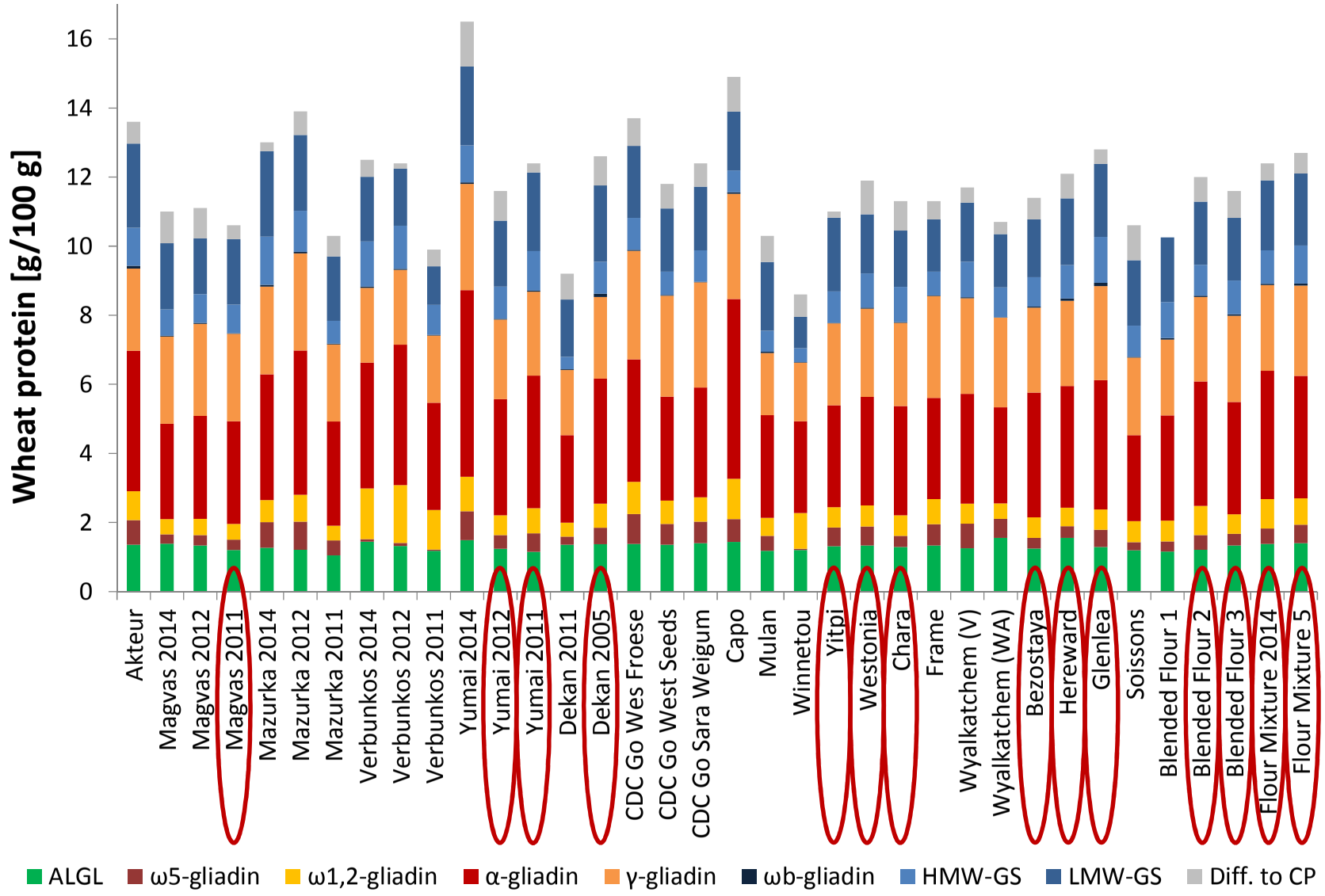
RP-HPLC of wheat flours



Quanti-tative criteria fulfilled

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Deutsche Forschungsanstalt für Lebensmittelchemie

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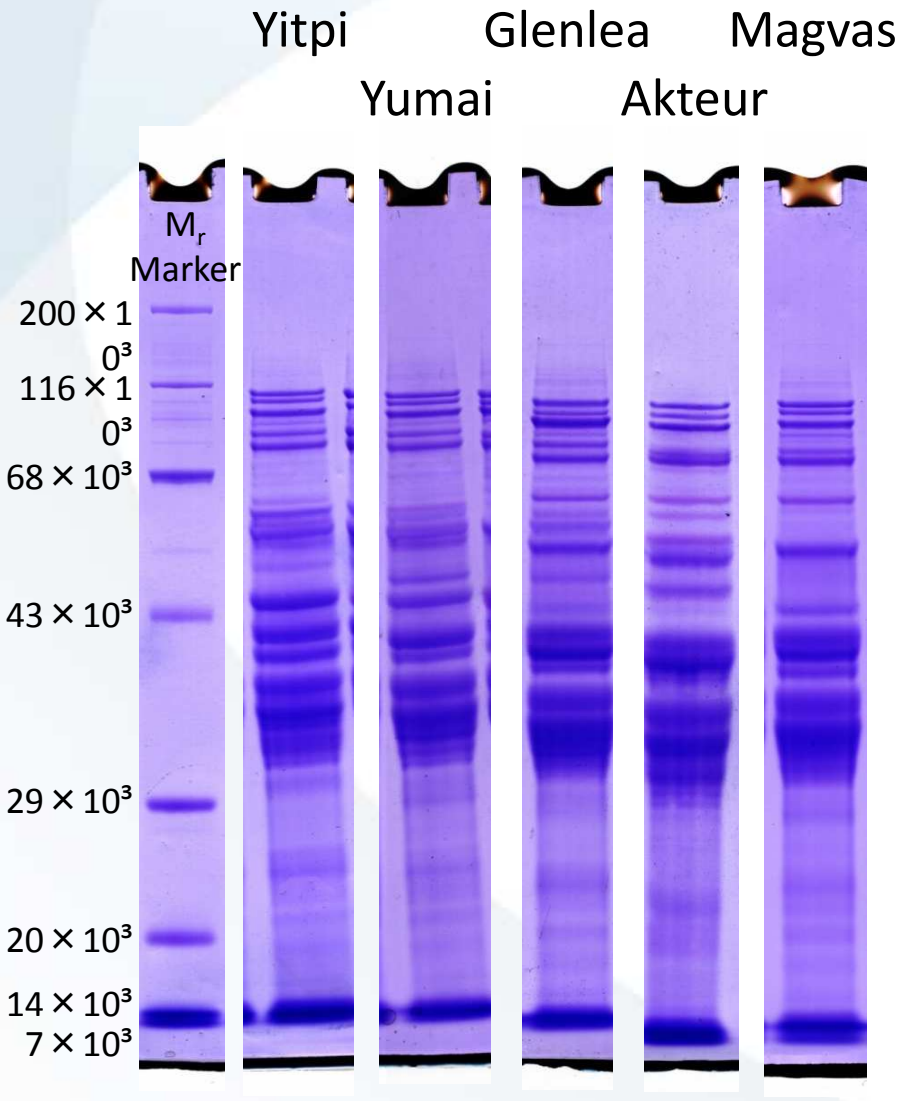
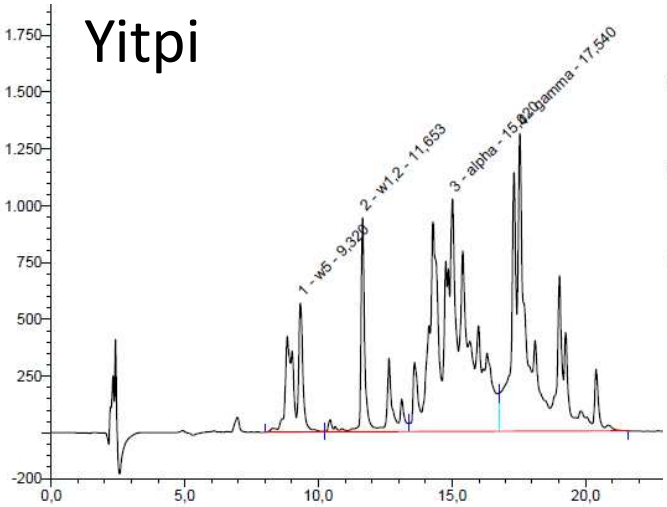
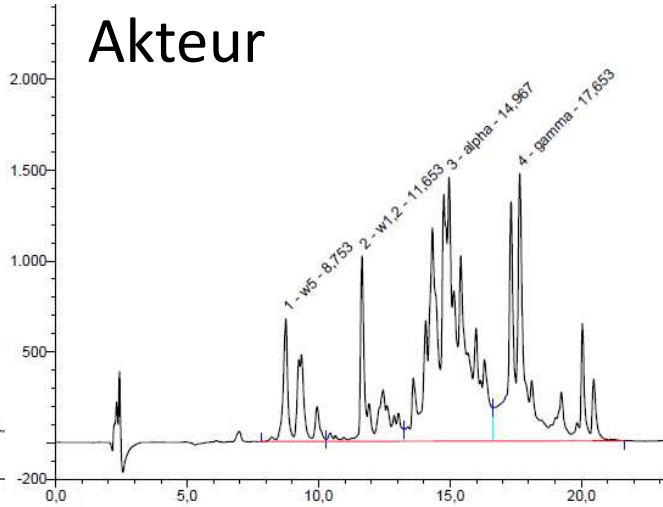
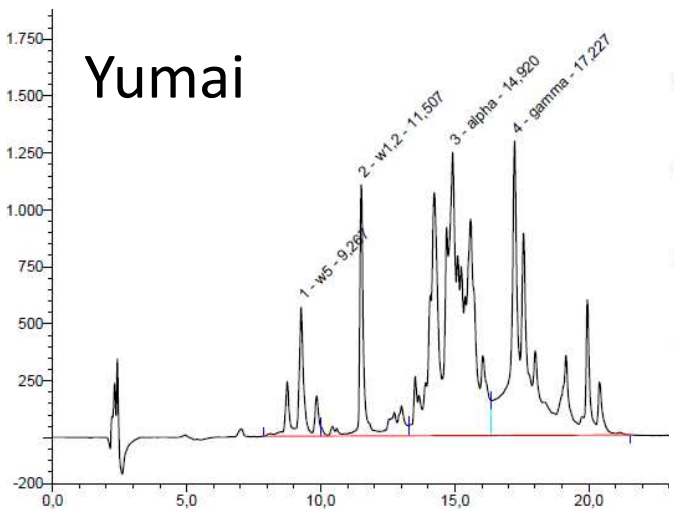
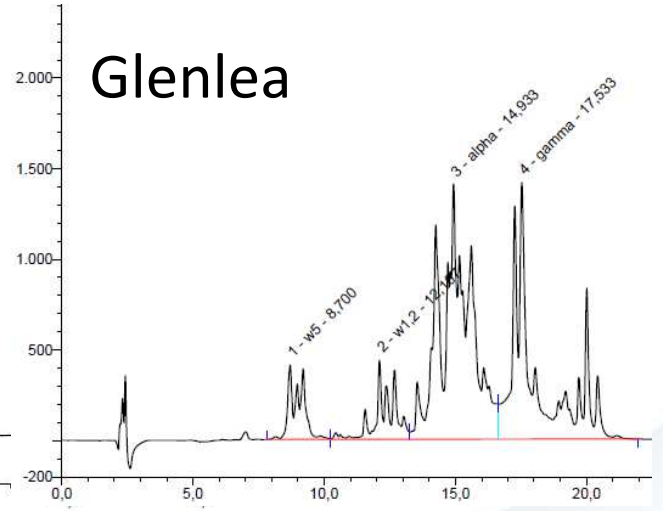
1D SDS-PAGE + RP-HPLC (Gliadins)











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Summary

- **Reference Materials** are needed for food allergen and Gluten-free analysis
- An **international consortium** led by MoniQA took the initiative
- **Dried skim milk** is available, characterized for food allergen analysis
- **Blank** matrix material based on a gluten free rice cookie is available
- **Different concentrations** of milk in a rice cookie (incurred) to become available by the end of 2015: 0, 5, 10, 50 ppm SMP
- **Inter-laboratory testing** of milk and blank in October/November 2015
- **Wheat and gluten free** materials being characterized, first availability of materials in 2016

Food and Health – Risks and Benefits

Innovative Technologies for Food Quality and Safety Management

Next Task Force Meeting: prior to PWG in Tulln, Austria
8 October 2015, 16:00-19:00

5th MoniQA International Conference 2015
16-18 September 2015, Porto, Portugal

MoniQA Association

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Get involved?!

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