



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Antibodies and their suitability for food allergen detection

Alergeny v potravinách a jejich diagnostika
27. duben 2012

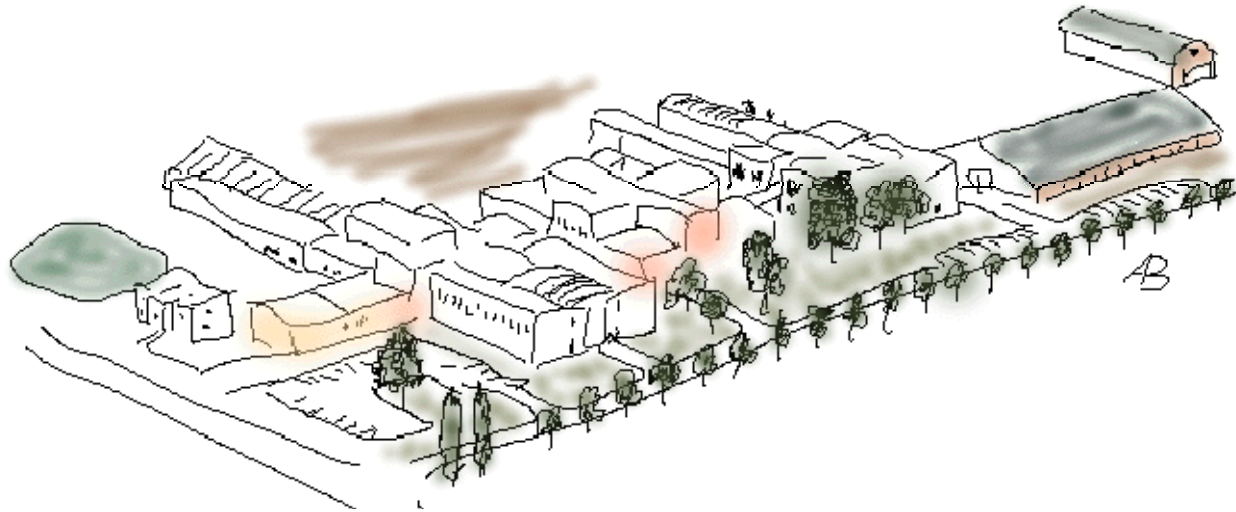


Universität für Bodenkultur
Wien

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für Agrarbiotechnologie Tulln

Sabine Baumgartner

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Antibodies and their suitability for food allergen detection



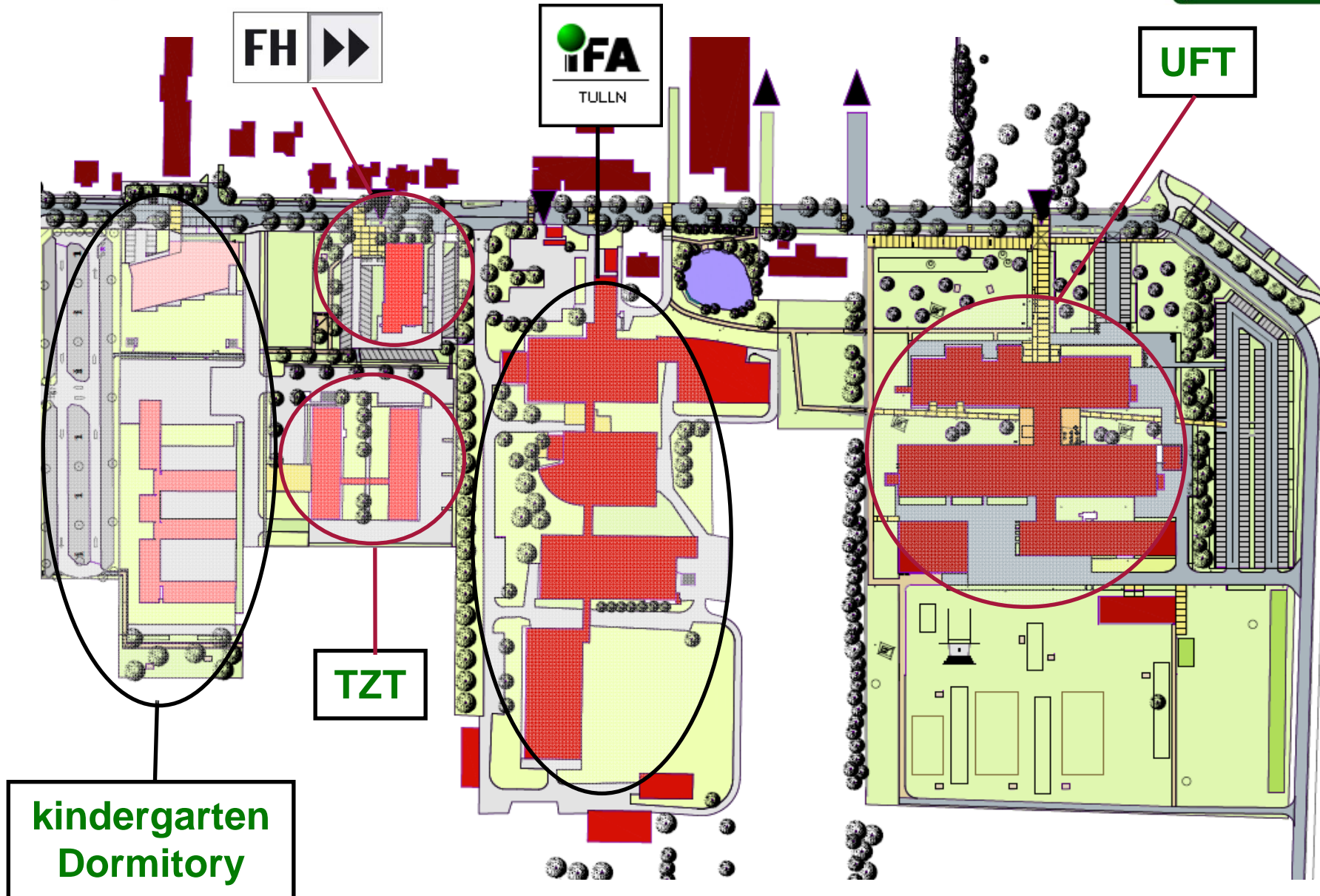
Sabine Baumgartner

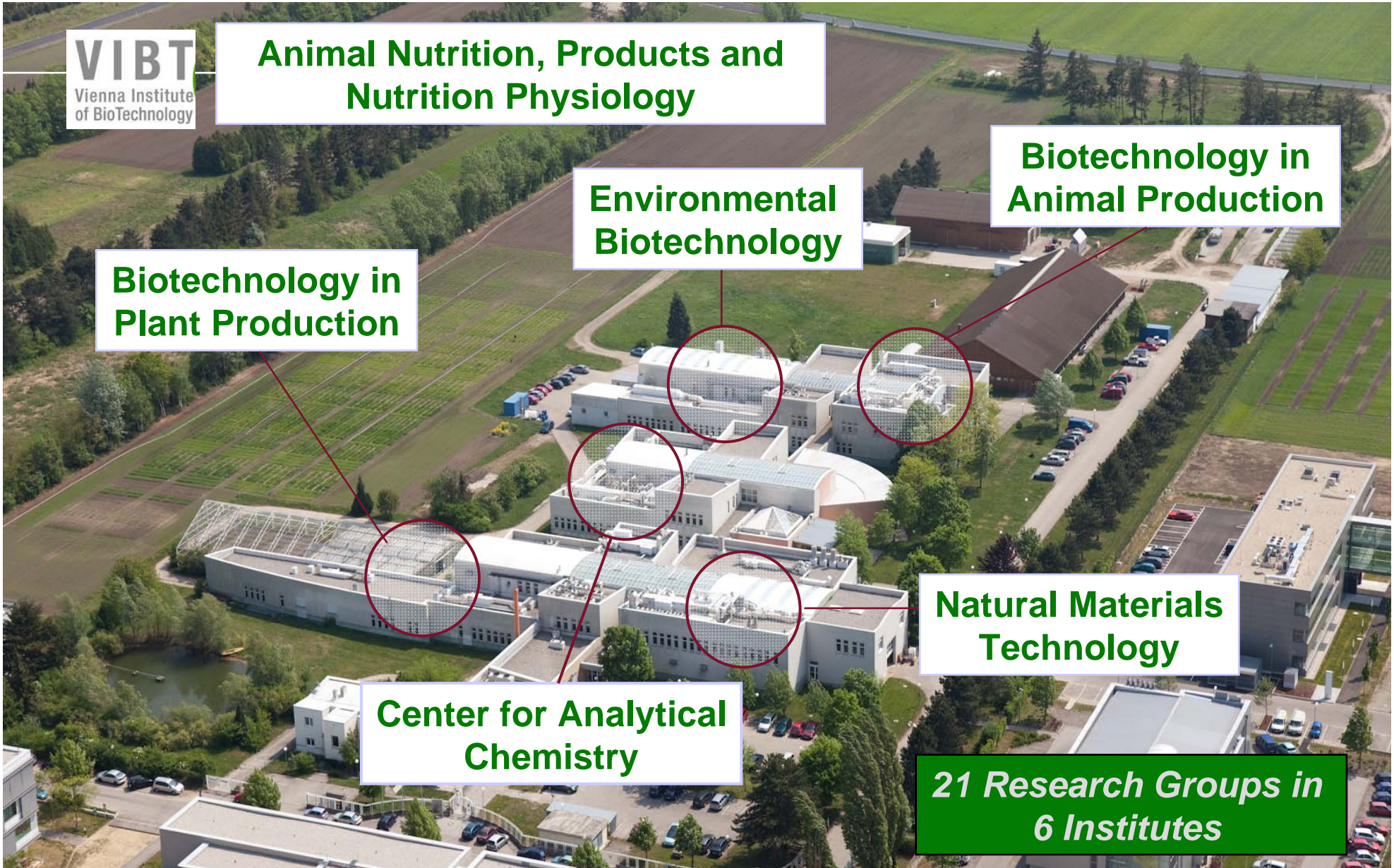
Christian-Doppler Laboratory for Rapid Test Systems for Allergenic
Food Contaminants

University of Natural Resources and Life Sciences Vienna,
Dept. IFA-Tulln, Center for Analytical Chemistry
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Campus Tulln 2011





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Vienna Institute
of BioTechnology

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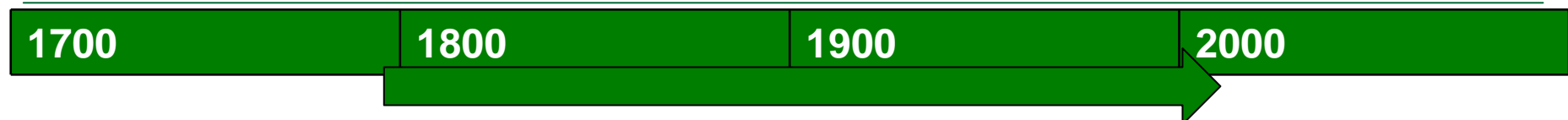
BOKU

Needle in the haystack – food allergens in food



Timeline of Immunology

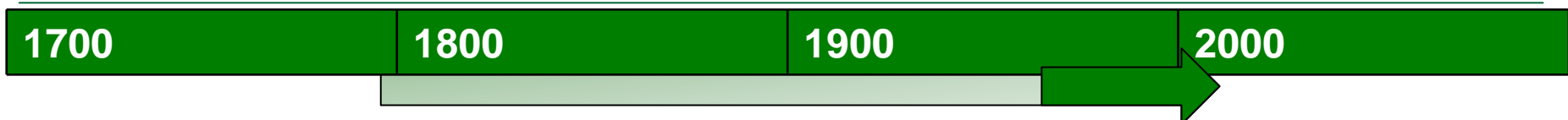
- **1798 – Jenner - vaccination small pox**
- 1883 – 1905 development of cellular theory on immunity
- 1890 – Von Behring/Shibasaburo Beginning of humoral theory of immunity
- **1900 – Ehrlich Antibody formation theory**
- **1917 – Landsteiner Hapten**
- **1938 – Marrack Antigen-Antibodybinding hypothesis**
- 1942 – Landsteiner/Chase Anaphylaxis; Freund Adjuvants
- **1948 – Antibody production in B-cells**
- 1964-68 T and B cell cooperation in immune response
- 1965 – Secretory immunoglobulins
- 1967 – Ishizaka Identification IgE
- **1971 – Engvall/Perlmann – ELISA**
- 1972 – structure of antibody molecule
- **1975 – Köhler/Milstein generation of first monoclonal antibody**
- 1980 - ...IL receptor discoveries, T-cell receptor gene identification, Th1 vs Th2 model, T-cell activation, gene therapy, identification of toll-like receptors, vaccine development



History of ELISA

- **1960** – Yalow/Berson First described radioimmunoassay
- **1966** – Avrameas/Pierce linkage techniques Ab-enzyme; Wide/Porath immunosorbent technique
- **1971** – Perlmann/Engvall and Schuurs/Van Weemen papers published to perform ELISA/EIA
- Since now development of ELISA-like techniques utilizing fluorescence, electrochemiluminescence, real time PCR-reporters
- **1980** – fully automated test instrumentation mainly diagnostic immunoassays
- **1990** - first commercial available ELISAs for food allergen testing

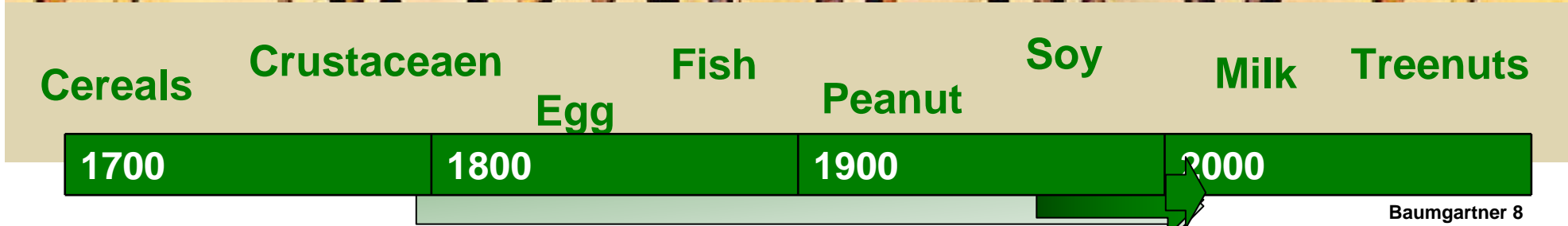
Lequin, 2005 Clin.Chem. 51, 2415-2418



History of Food Allergen Labelling in the EU (2000/13/EC, 2003/89/EC, 2006/142/EC, 2007/68/EC, 1169/2011)

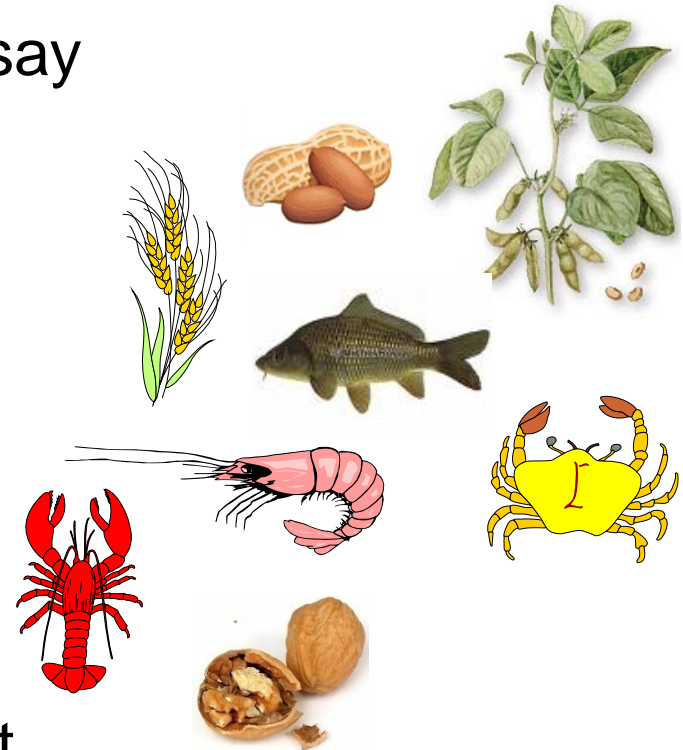


Celery, mustard, sesame, lupine, molluscs



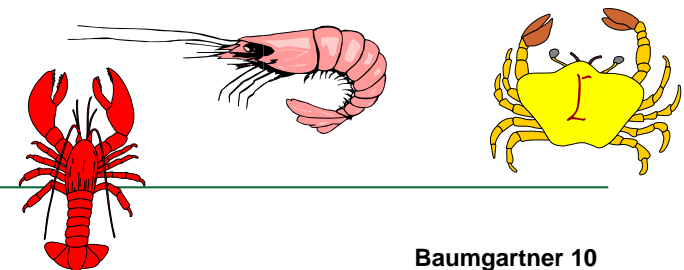
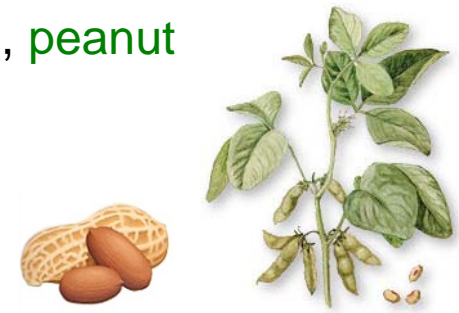
Demanding Food Allergen Detection

- Detection of allergenic proteins in food, mainly processed
- Detection - Proteinassay or Allergenassay
 - Extractable proteins
 - Markerproteins
 - Allergenic proteins
- Sources different protein families
- Protein specific extraction methods
- Characterisation, Validation
- No reference materials
- Reference methods under development



Protein classes

- „Pathogenesis-related“ Proteins – PR-Proteins
 - Chitinases, Proteases, antifungal proteins, Bet v 1 homologes, Amylase inhibitors, Peroxidases, fruits (cherry, apple,..), barley, rye
- **Profilins**
 - Actin-binding proteins, signalling: Celery, hazelnut, soya, **peanut**
- **Parvalbumin**
 - Ca²⁺-binding protein: cod, carp, salmon
- **Tropomyosin**
 - Actin-binding protein: Shrimps, lobster, crab



Protein classes

■ Seed storage proteins

- Albumins (2S), Vicilins (7S), Legumins (11S), Glycinin (11S), β -Conglycinin (7S): **peanut**, walnut, brazil nut, mustard, soya

■ LTP-Proteins (Lipid transfer proteins)

- Lipidmetabolism: apple, peach, plum, corn

■ Proteases

- Proteolysis: papaya, pineapple, kiwi

■ Peroxidases

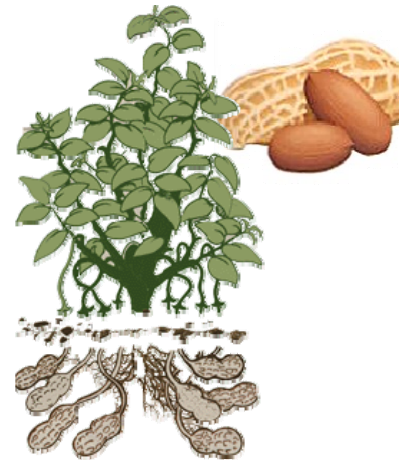
- Peroxidation: wheat, barley

■ Lectins

- Carbohydrate binding: **peanut** agglutinin



Peanut and products thereof



■ Composition

[%]	Protein	Lipid	CH	Fibers	Minerals
Peanut	27,4	50,7	9,1	7,5	2,7

Albumins
2S
Water soluble

Globulins
7S, 11S
Salt soluble

Prolamins
Soluble in
alcohol/water

Peanut and products thereof

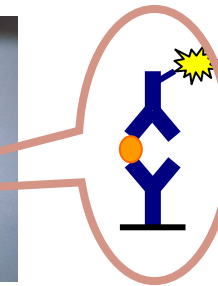
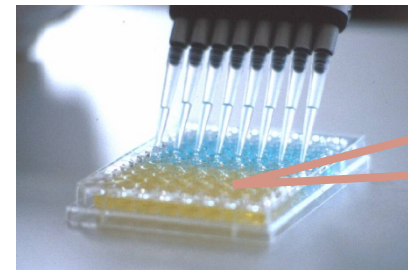
■ Allergens

- Albumins (2S) or Conglutinins: Ara h 2, 18 kDa, Xreacts - Ara h 6
Ara h 6, 14 kDa, X - Ara h 2
- Vicilins (7S): Ara h 1, 71 kDa, Xreacts - walnut (Jug r 2), pea (Pis s 1)
- Legumins or Glycinins (11S): Ara h 3, 14 kDa, Xreacts – Soya
Ara h 4, 61 kDa
- Profilins (Bet v 2 – Homologue): Ara h 5, 14 kDa, Xreacts - gras (Lol p 12),
birch pollen (Bet v 2)
- PR-Proteins: Ara h 8, 17 kDa, Xreacts – birch pollen (Bet v 1)
- LTP-Proteins: Ara h LPT, Xreacts – peach (Pru p 3)
- Oleosins: Ara h Oleosin, 17 kDa
- Agglutinins: Ara h Agglutinin, 29 kDa



Possible methods

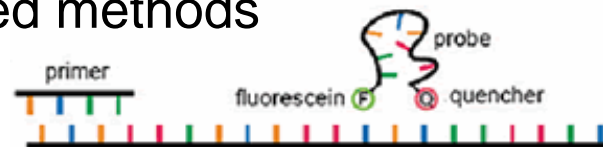
- Immunochemical methods
 - ELISA
 - Rapid tests – Lateral Flow Devices, Dipsticks



© Barbara Cvak

- DNA-based methods

- PCR
- rt-PCR
- PCR-ELISA



- Upcoming MS-methods



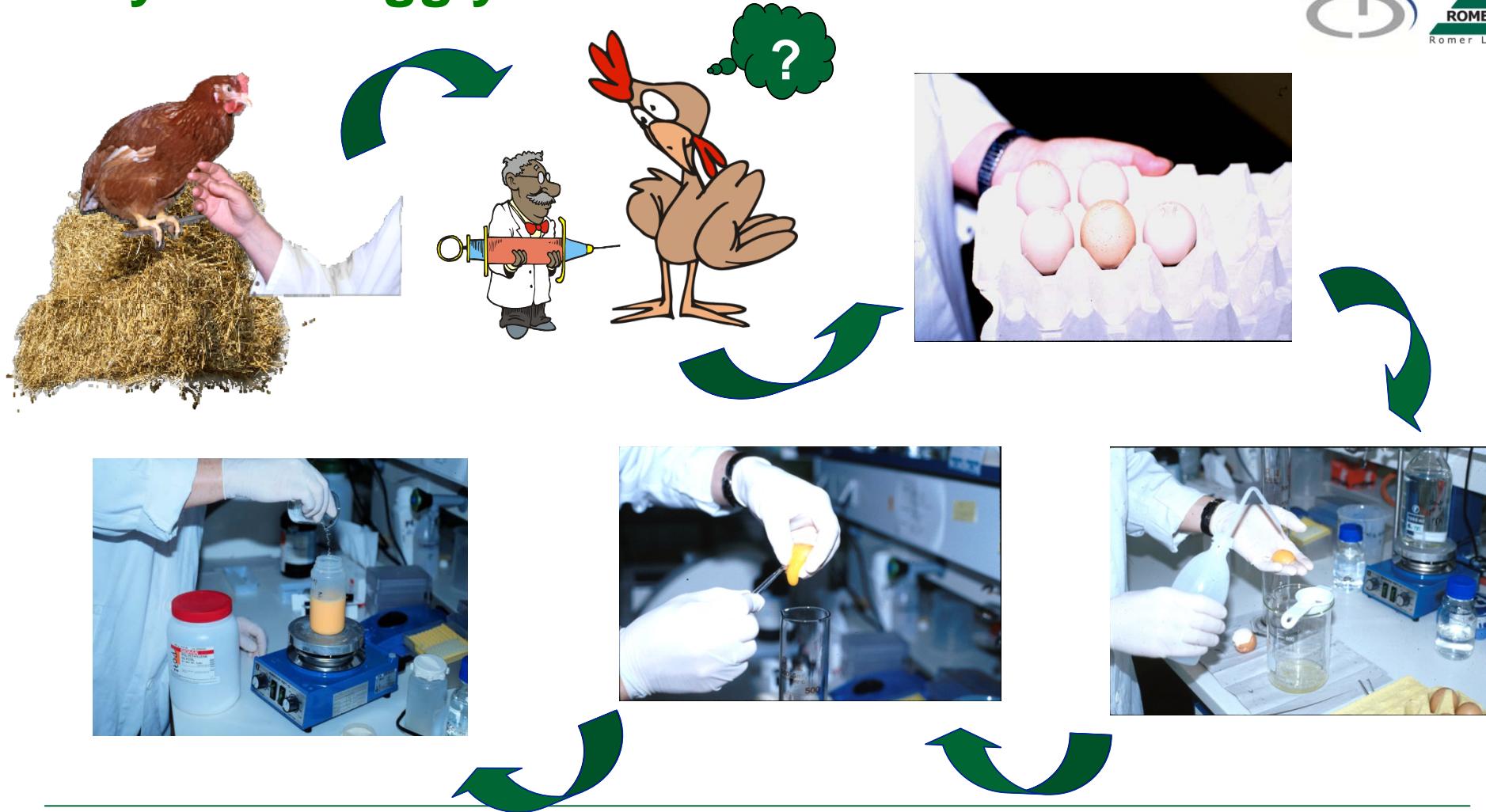
Immunochemical methods - Antibody based assays



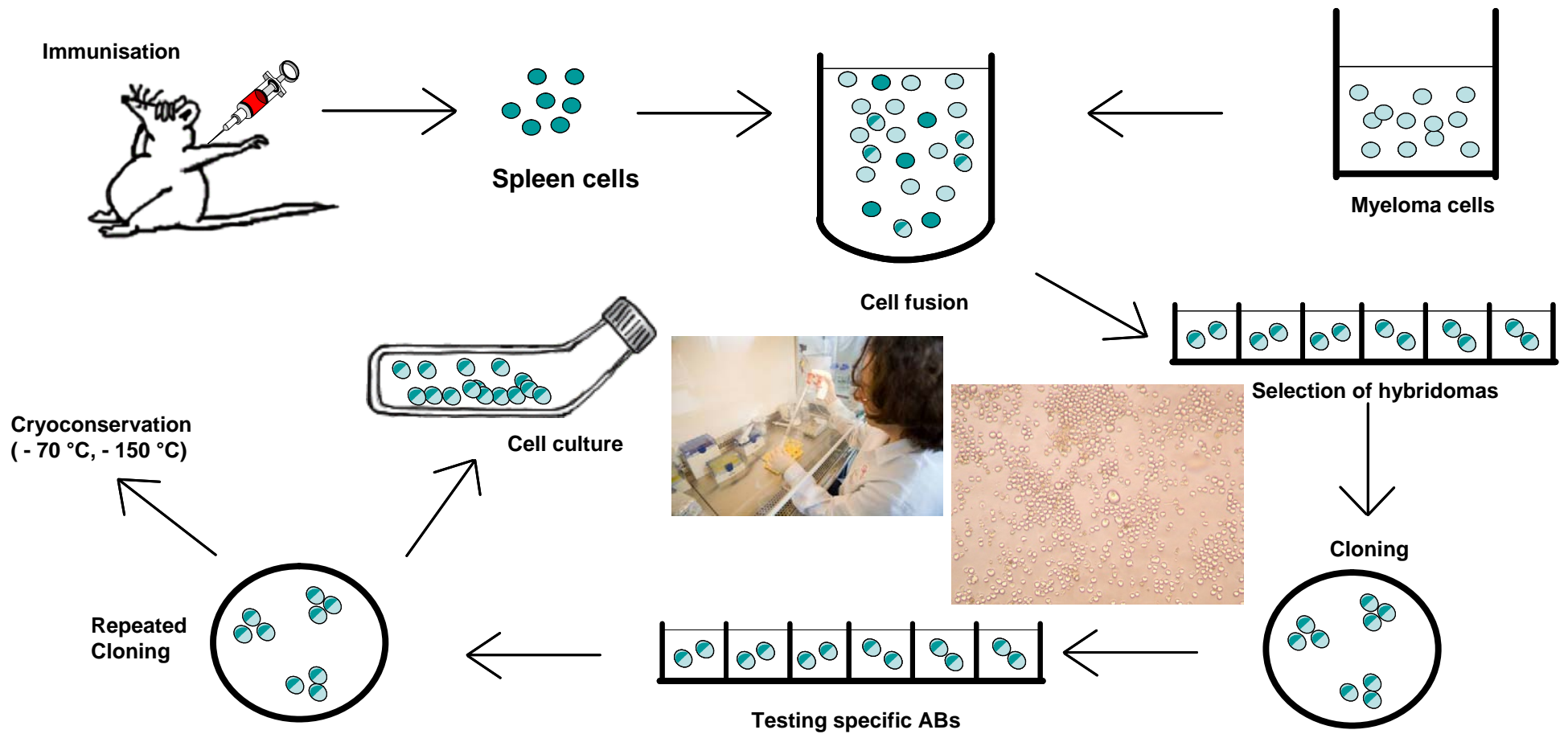
- Protein detection by highly specialised proteins
- Polyclonale Abs (rabbit, chicken, sheep,...)
- Monoclonale Abs (mouse, rat,...)
- Recombinant Abs



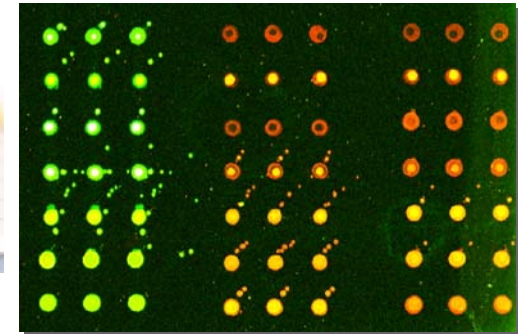
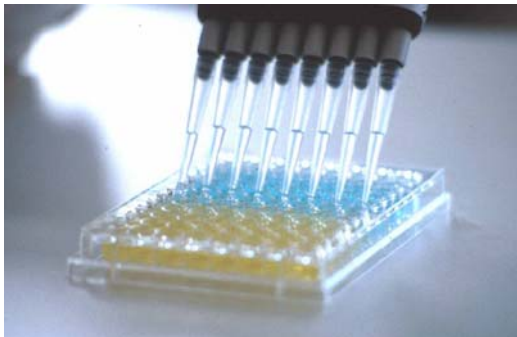
Polyclonal egg yolk antibodies



Monoclonal antibodies



Immunoanalytical Solutions for Food Allergen Detection



Microtiterplates ELISA's	Lateral Flow Devices	Biosensors	Micro Arrays
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96 wells

1 Assay

1 Assay

Hundreds

½ h – 3 h

Minutes

Minutes

Minutes

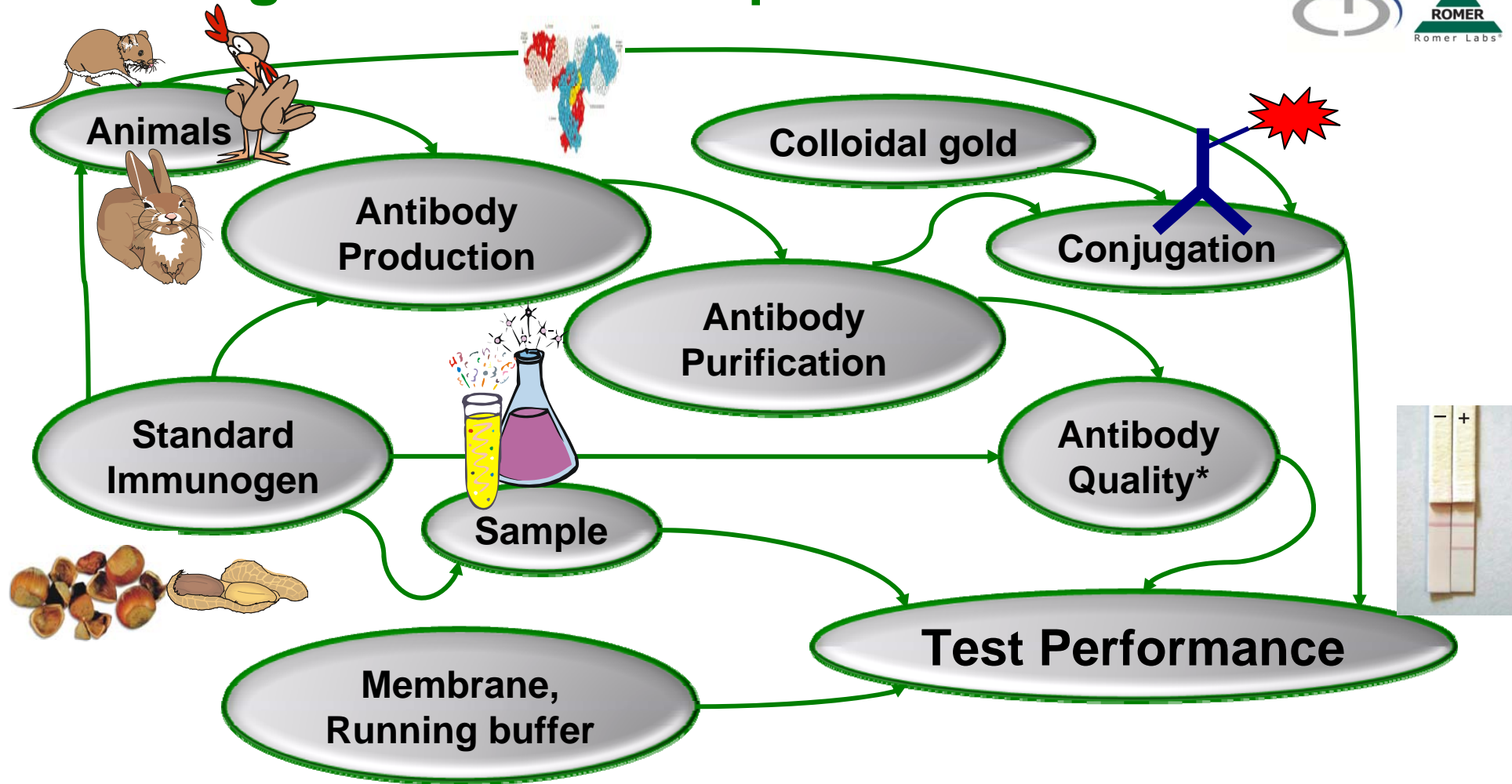
Lab

on-site

Lab/on-site

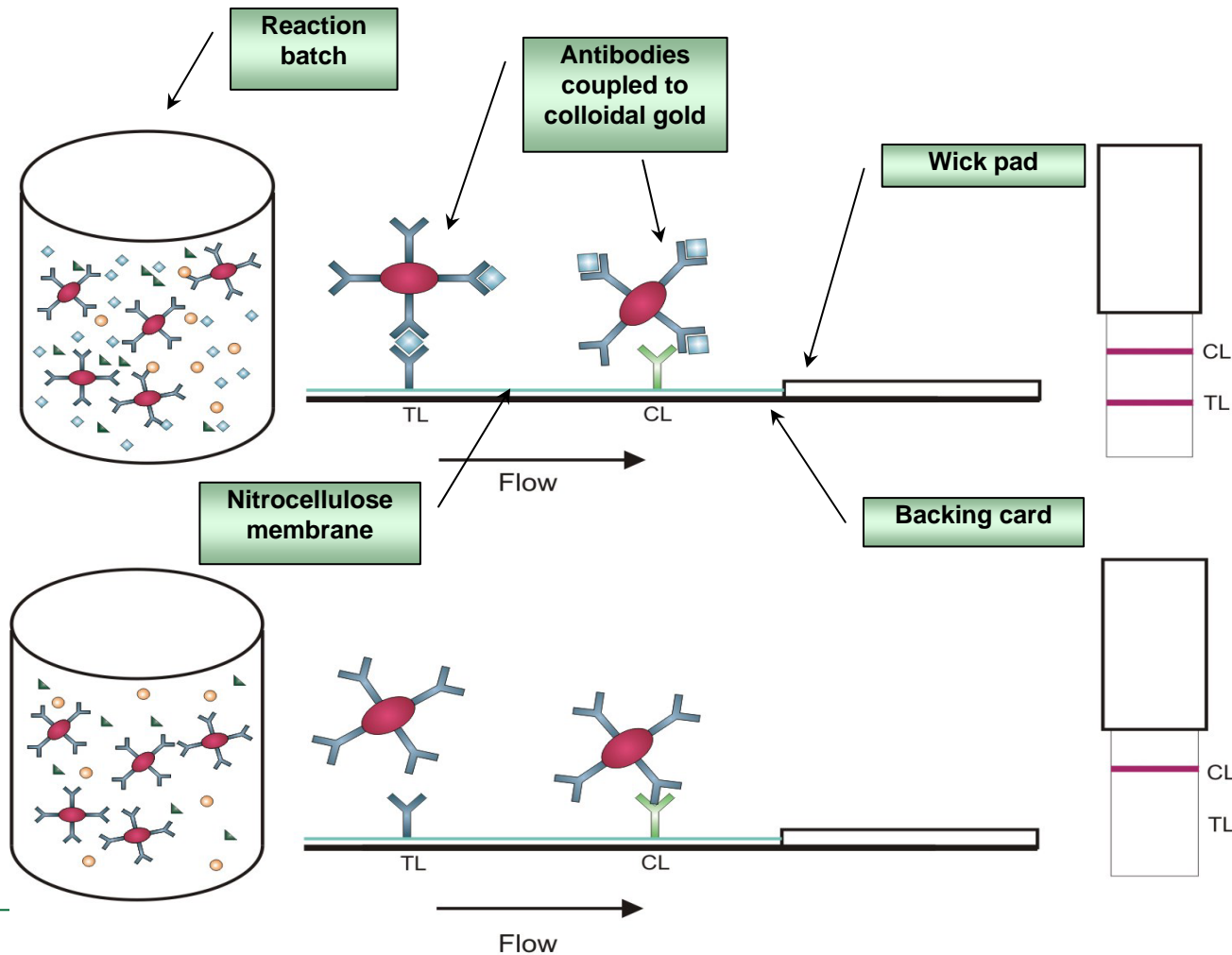
Lab

Challenges in LFD development



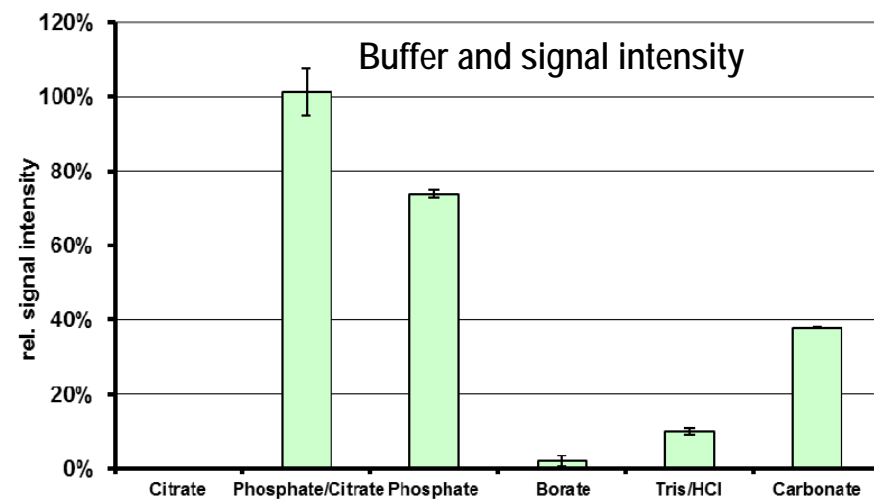
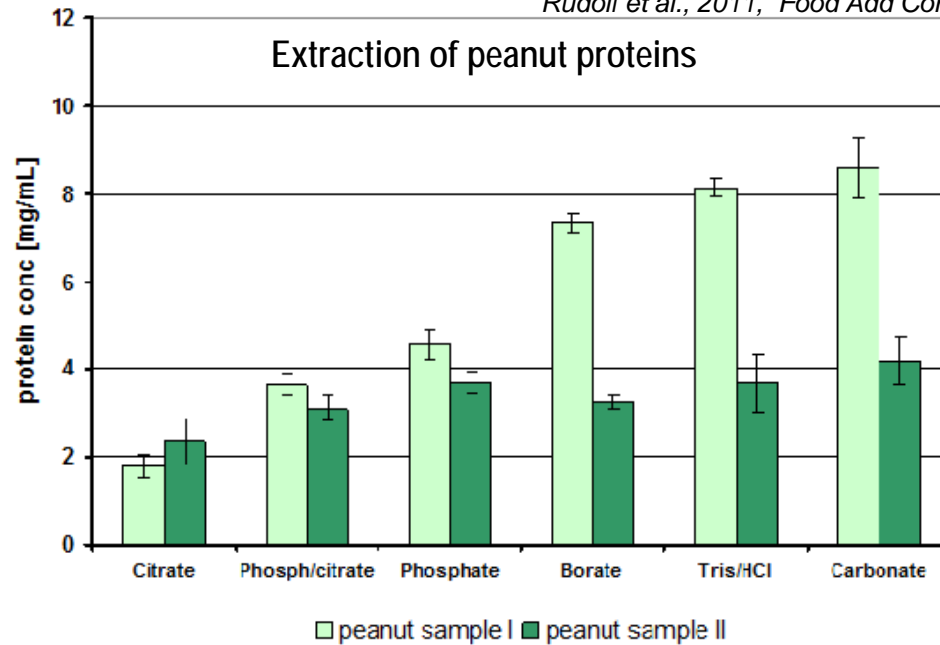
* sensitivity, specificity, yield

LFD principle – Sandwich format

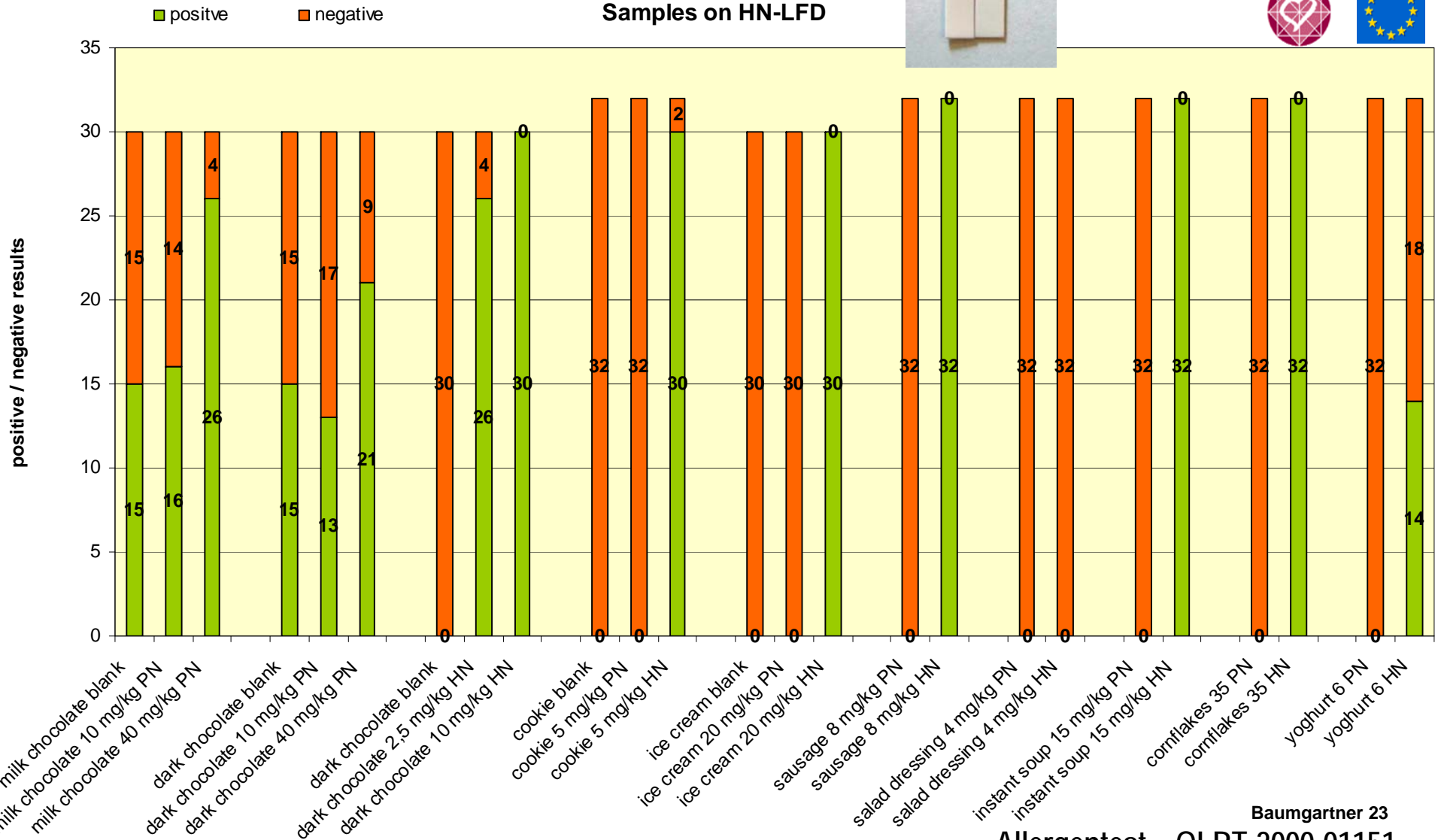
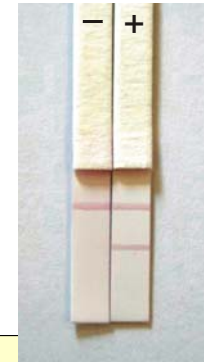


The extraction - example peanut

Rudolf et al., 2011, Food Add Contam



LFD – Hazelnut determination



Something new?

	ELISA 2007	ELISA 2012	LFD 2007	LFD 2012	PCR 2007	PCR 2012
Cereals	X*	X*	X	X	X	X
Crustaceans	X	X	X	X	X	X
Eggs	X	X	X	X	X	?
Fish	?	?	?	?	X	X
Peanuts	X*	X*	X	X	X	X
Soy	X	X	?	X	X	X
Milk	X	X*	X	X	?	?
Treenuts	X	XXX*	X	XXX	X	XXX
Celery	X	?	?	?	X	X
Mustard	X	X	?	X	X	X
Sesame	X	X*	?	X	X	X
Lupine	X	X	?	X	X	X
Molluscs	?	X	?	?	X	X



Example peanut – Kit comparison

	Hurst et al., 2002	Koch et al., 2003	Poms et al., 2005 30 partners EC-ring trial
Number of Kits	4	3	5
Matrix	Milk and dark chocolate	Cookies	Cookies, dark chocolate
Measurement range	10-200 mg/kg	500-2500 mg/kg	2-10 mg/kg
Recovery	3-250%	30-90%	44-191%

Conclusion

-what makes it so sophisticated?
 - Find the appropriate analyte
 - Marker
 - Allergen
 - Whole protein
 - Referencematerials
 - Referencemethods
 - Thresholds/Limits
 - Nearly unlimited preparation and processing possibilities for food!!



XIth ICAFA

- XIth International Conference on AgriFood Antibodies
- Ibis Wien Mariahilf Hotel, Wien
- 3rd - 5th September 2012



St. Stephen's Cathedral, Vienna

- <http://sites.google.com/site/icafaconference/chain-act-10>
- Dates for the Diary
 - 7th July 2012: Early registration and payment deadline
 - Download registration form:
<http://sites.google.com/site/icafaconference/album>
- Contact Prof. Chris Smith:
 - c.x.smith@mmu.ac.uk

The funding and the people behind



Christoph Hasenhiendl



Brigitte Galler



Parisa Ansari



Denise Huber



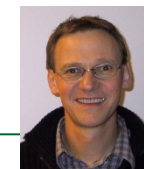
Manuela Führer



Norbert Stoppacher



Judith Rudolf



Thank you for your attention!



Same but different!

