

Research in a routine lab

problems and opportunities

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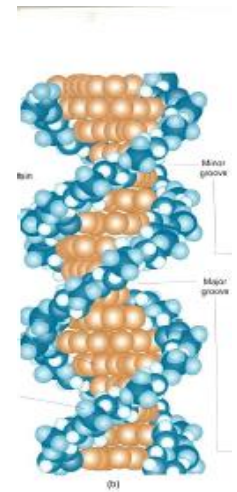
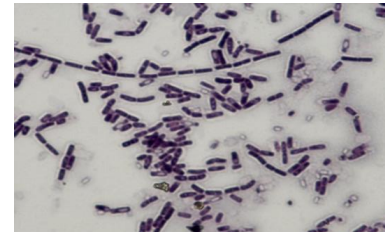
Laboratoire National de Santé – Service de Surveillance Alimentaire

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EFSA focal point Luxembourg – November 26, 2019

Service de Surveillance Alimentaire – activities

- Microbiological analysis (Salmonella, Listeria, E-coli, Bacillus cereus, EHEC,...)
- pesticides in food of animal and vegetable origin
- mycotoxins in cereal-based food and feed
- additives (colourants, sweeteners, preservatives)
- agricultural contaminants (nitrates, nitrites)
- process contaminants: furan, acrylamide, MCPD, PAHs
- food contact materials (FCM)
- GMO - detection and quantification of genetically modified organisms
- allergens using ELISA and PCR technology



Service de Surveillance Alimentaire – NRL

- 1 - dairy products
- 2 - pesticide residues in cereals
- 3 - pesticide residues in fruits and vegetables
- 4 - pesticide residues – ‘single residue methods’
- 5 – process contaminants
- 6 - mycotoxins
- 7 – plant toxins
- 8 - food contact materials
- 9 - genetically modified organisms (official control)
- 10 - genetically modified organisms (validation of methods)
- 11 - Salmonella *
- 12 – EHEC * (enterohaemorrhagic E. coli)



Part of the European Network of GMO Laboratories (*ENGL*)

* together with the Microbiology Laboratory of the National Health Laboratory and the Laboratory for Veterinary Medicine

Service de Surveillance Alimentaire – official missions

1 - L'établissement a pour objet:

de développer des activités analytiques et d'expertise scientifique liées à la prévention, au diagnostic et au suivi des maladies humaines;
d'assurer le rôle d'un laboratoire national de contrôle ou de référence;
d'assurer des missions à caractère médico-légal.

2 - L'établissement contribue au développement, à l'harmonisation et à la promotion des méthodes et techniques de laboratoire, en étroite collaboration avec les laboratoires d'analyse du pays et de l'étranger.

3 - Dans le cadre de ses attributions, l'établissement développe des activités de recherche et d'enseignement.



R&D activities strongly encouraged by EURLs

Loi du 7 août 2012 portant création de l'établissement public «Laboratoire national de santé»

Benefits of research

- Staying « up to date » with scientific developments (new methods, new techniques, new findings, upcoming issues, etc).
- Innovation: getting familiar with new techniques (i.e. ASE, SPME, etc.) and implement them into our laboratory
- Being pro-active: implement and validate methods before the need for the stakeholders is there
- Scientific publications in peer-reviewed journals: increase of reputation of the laboratory (national and international)
- Becoming attractive for (international) laboratories for networking and collaborations
 - recent collaborations with
 - LUA Saarbrücken
 - BfR and BVL Berlin
 - BIOR Latvia

Obstacles for OCLs / routine labs

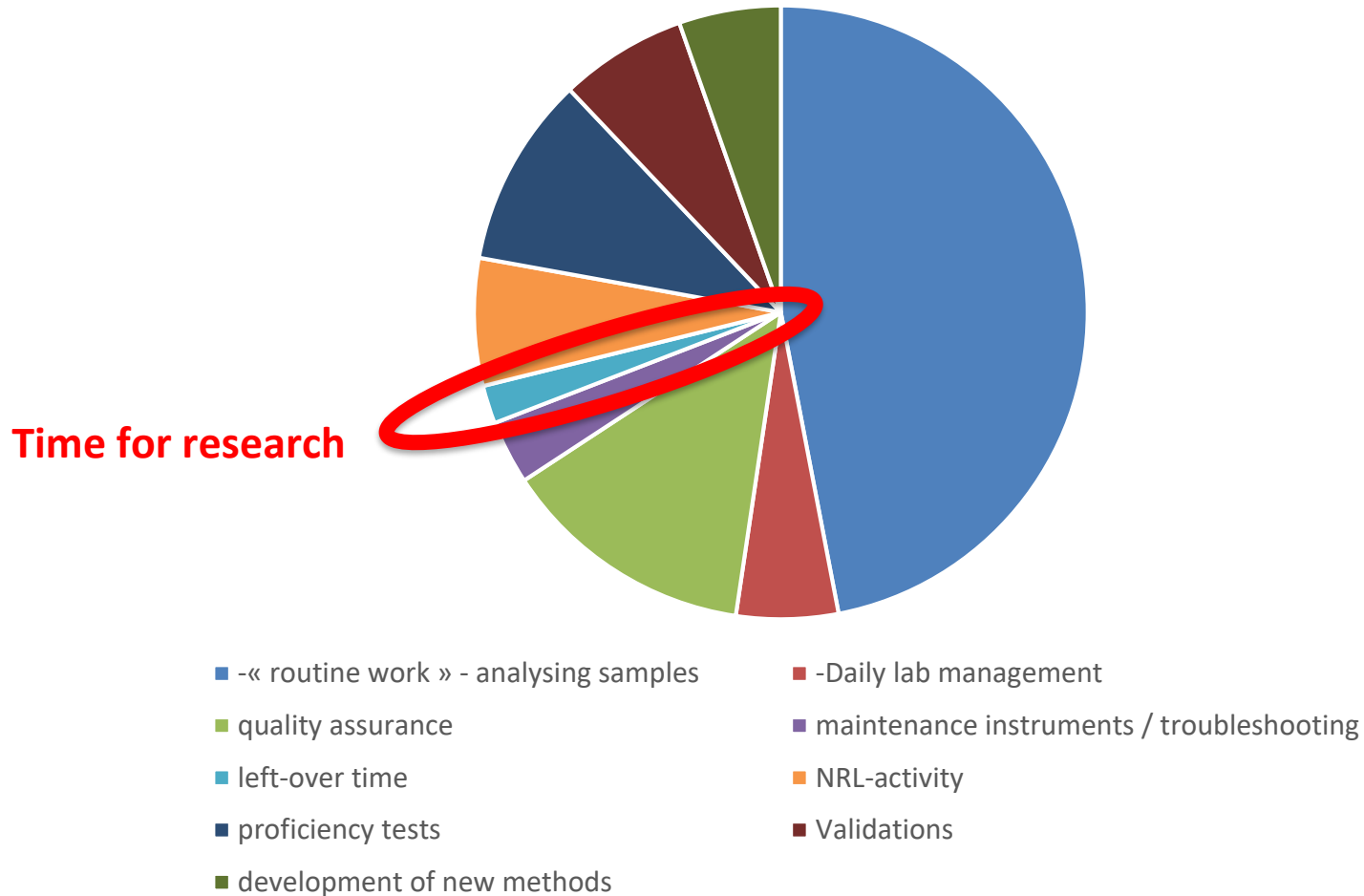
- Routine activity is always first. Research activity is secondary and dependent on availability of scientists and technicians

Routine activity includes:

- « routine work » - analysing samples
- Daily lab management
- Development of new methods asked by EURLs or stakeholders
- Validations
- Maintenance and troubleshooting of instruments
- NRL-activity (networking, communication with EURLs, traveling)
- Proficiency tests (> 50 / year)
- (increasing) quality control management in order to keep accreditation (implication of all lab staff according to ISO 17025:2017)

Obstacles for OCLs / routine labs

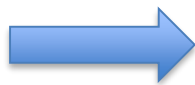
Expenditure of time



Obstacles for OCLs / routine labs



- Financial resources: research activities not covered by routine budget

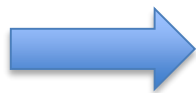


Participate in national or international project calls in order to increase the research budget?

-> not possible because follow-up requirements (timelines, intermediate reports, etc) cannot be guaranteed

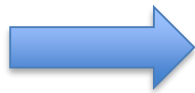
Advantages of routine labs compared to pure research labs

- Major problem of many research labs: where to get samples ?



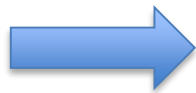
Our routine lab: > 4000 samples per year.
(have to be rendered anonymous)

- Pressure to achieve results and publish in high-ranked peer-reviewed journals?

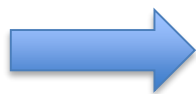


Research is a secondary activity, no results are expected.
Publications are a « bonus »

- No streamlining of research topics, no (financial) pressure to investigate « popular » themes



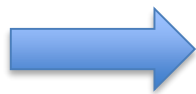
Freedom of choosing research topics even though they are isolated and don't allow follow-up studies



Being innovative, investigate a priori non-promising tracks, as failing is not dramatic, as the survival of the lab doesn't depend on the research outcome

Our solutions

- Solution of time / availability problems



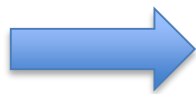
MSc students with internships of 4 – 6 months: usually well-trained and highly motivated.

- Restrictive budget for standards and / or reagents



Accreditation doesn't allow us to work with (slightly) expired chemicals -> Use them for research [*but confirm accuracy*]

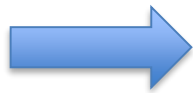
- Research topics



- Implement new method and push the validation over target
- Start from abnormalities observed in routine analysis and follow-up

Our solutions - outcome

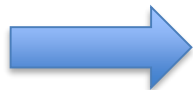
- Presentations at EURL-NRL workshops



- Collaborations with EURLs, start collaborations with other NRLs

- strengthen our position as NRL in LU (gain experience and expertise) and in the respective networks (reputation, attractiveness for collaborations)

- Publications in peer-reviewed journals (1-2 per year)



- help LNS in its performance contracts

Recent research projects

PAHs in smoked tea

CONTEXT: IMPLEMENTATION OF METHOD IN LAB AND APPOINTMENT NRL

Background of the study

- Tea is 2nd most consumed beverage in the world
- Tea leaves contaminated with PAHs during drying
- **Some special kinds of teas (Mate, Lapsang Souchong) are smoked over bamboo fire**



Migration of PAHs into infusions ?

Results

- High PAH concentrations in smoked tea leaves (27 – 220 µg/kg for sum of PAH4)
- High migration rates into infusions: > 80 %
- However final concentration remain low (highest: 2.7 µg/L for sum of PAH4)

One of the starting points of a current PhD-thesis at Sciensano (Brussels, BE)

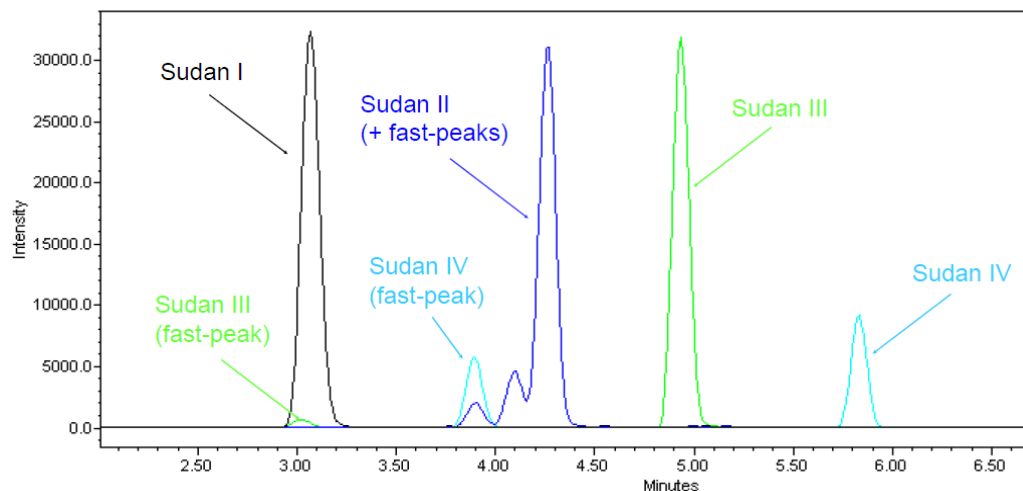
Recent research projects

Sudan dyes in spices

CONTEXT: OBSERVATION OF « FAST » PEAKS

Background of the study

- Sudan dyes are synthetic fat-soluble azo-compounds (-N=N-) of bright red colour
- Fraudulently added to spices, palm oils, etc, in order to intensify the (red) colour



Results

- **Fast peaks may lead to false negatives or underestimations**
- Identification of conditions causing fast peaks (used column, matrix interferences)
- Proposition of new clean-up steps in order to avoid interferences

Recent research projects

Phthalates in Beer

CONTEXT: IMPLEMENTATION OF « SPME » IN THE LABORATORY

Background of the study

- Develop an SPME-method for phthalates in beer and thus shorten time and accuracy of the analysis
- Realise monitoring of phthalates in local beers
- Investigate influences of storing containers: cans, glass bottles and aluminum bottles

Results

- Method in use in our laboratory. Feed-back of other laboratories that adapted their own method according to our findings
- DEHP detected in 93 % of local beers, though at low concentrations (max 1.5 µg/L)
- No conclusion about the influence of storage container. Difficult to determine exact origin of contamination (contaminated cereals, brewing process, bottling, etc...)

Recent research projects

Ergot alkaloids (I) - Monitoring

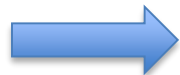
CONTEXT: IMPLEMENTATION IN THE LABORATORY

Background of the study

- Develop a fast UHPLC-method in order to satisfy the needs of our stakeholders and EFSA in terms of sensibility
- realise assessment of ergot alkaloid contaminations of different types of cereals (rye, wheat, barley, etc.) grown in Luxembourg

Results

- Accreditation of a method analysing all 6 alkaloids with their epimers in 15 minutes. LOD = 1 µg/kg; LOQ = 5 µg/kg
- Highest average concentrations measured in rye (> 500 µg/kg for the sum of 6)
- Highest absolute concentration measured in wheat (>2500 µg/kg for the sum of 6)



Proof of necessity not to limit monitoring to rye

Recent research projects

Ergot alkaloids (II): Influences on the epimerization of ergot alkaloids

CONTEXT: DEMAND OF EURL-PT: QUANTIFY INDIVIDUAL EPIMERS

Background of the study

- For conformity testing, limits about ergot alkaloids in food will be introduced for the sum of all alkaloids together with their epimers, however for monitoring and risk assessment it might be interesting to analyse the epimers separately, because The R-form is more toxic than the S-form
- Epimerization occurs spontaneously, so it is important to understand epimerization in order to avoid epimerization in the lab to give correct results for each epimer

Recent research projects

Ergot alkaloids (II): Influences on the epimerization of ergot alkaloids

Results

- Heating, humidity, pH and UV-light were all found influencing epimerization. Strongest results are obtained with the combination of humidity and heat for about 1 hour -> **baking is confirmed to favour epimerization and to decrease concentrations**
- No complete change into 100 % « S » was possible
- Ergotamine and ergosine seem not to do significant epimerization
- Heat and UV-light seem to favour degradation of some alkaloids (e.g. ergokryptine and ergocornine) but not for all of them
- The epimerization seems to be matrix-dependent

Ongoing research projects

On-going or planned innovative approaches through molecular biology techniques

- Multiplex-real-time PCR for simultaneous allergen detection
- Monoplex-real-time-PCR for detection of marzipan adulteration with apricot/peach kernels
- DNA-bar-coding approaches for species detection: mitochondrial (e.g. COI) regions for animals, chloroplastic (*matK* and *rbcl*) regions for plants, ITS2 regions as alternatives
- High-resolution DNA melting profiles for detection of aromatic herbs adulteration
- Analysis of the relationship between the presence of cereulide (by UPLC MS/MS) and its coding gene (*ces*) in presumptive *Bacillus cereus* strains isolated from routine food samples (by HRM-Real-Time-PCR)

Thank you for your attention !