



A context-based learning (CBL) resource

Facilitator version

Dylan P. Williams, Katy J. McKenzie, David L. Davies, Khalku Karim, Elena Piletska & Kevin Parker

University of Leicester & KKI Associates Ltd

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Introduction

This resource is designed as a contextualised introduction to the application of several key chemical concepts (eg analytical chemistry) to an area of industrial activity.

The problem has been designed to be used with small groups of undergraduate students (ideally of 4–6 members) studying chemistry, natural sciences or biochemistry at year one or year two level. The scenario places the groups in the roles of undergraduate students during an industrial placement at a food analysis laboratory. The assessment of this problem integrates a number of different components. Students must work on a number of different types of submission which target different audiences including other professional scientists, local government authorities, the media and members of the general public.

Delivery of the problem

The module integrates a number of units which may be run separately or in series (in whatever order the tutor chooses):

- 1. The DNA and protein based analysis of meat fraud cases
- 2. An open ended laboratory investigation into the use of simple spectroscopic and chromatographic approaches to detect contamination in coffee
- 3. An open ended practical investigation based on the detection and extraction of toxins and contaminants

Criterion	Value
Intended level	Year 1–2 (Levels 4 & 5)
Subject area	Analytical chemistry, Molecularly imprinted polymers
Contact Hours	10–12 hours
Group size	4–6

Table 1: Module description

Tutor text has been included in this version of the problem. The tutor text in this document should not be shown to students.

Outline tutor answers have been provided for the facilitation questions. Please note that these are neither model answers nor guidelines to the amount of content that students should produce. These answers only provide a minimal outline of the concepts being asked and students should go into more detail and provide examples of each of these concepts.

We recommend that this problem is used with small groups of students (typically group sizes of 4 - 6 work best). We also recommend that each facilitator guides no more than 2 or 3 groups – if a facilitator has to work with any more groups than this, it is likely to mean that very little time is spent with each group.

We have found that postgraduate students can make good CBL facilitators (especially those who have experienced the approach as undergraduates) if they are given guidance in this style of teaching and the nature of the problem before the start of the module. It is advisable to have at least one staff facilitator on duty during all sessions.

Context based learning

This resource is based on a context based learning (CBL) approach. The CBL approach based on the presentation of learning activities in a context-rich format (Belt, et al., 2005) specifically in the form of case studies and group investigations. The CBL approach allows students to apply fundamental concepts in a meaningful context (which can improve student engagement). The approach also allows the integration of high level transferable skills training alongside the teaching of 'core' scientific concepts. For further information, please see the references below.

- Belt, S. T., Leisvick, M. J., Hyde, A. J., Overton, T. L. & Summerfield S. (2005), Using a context-based approach to undergraduate chemistry teaching – a case study for introductory physical chemistry. Chemistry Education Research and Practice, 6, (3), 166–179.
- Overton, T. L. (2007), Context and problem-based learning, New Directions, 3, 7– 12
- Belt, S. T., Davies, D. L., Martin, P., Moss, K., Overton, T. L., Symons, S. L., Williams, D. P. & Woodward, J. R. (2009) Context/Problem-Based Learning in Chemistry – Sharing Lessons Learnt & Making it Work. Royal Society of Chemistry, Available online: <u>http://rsc.li/1pkb24n</u> (accessed 29/04/2015)

Transferable skills development

This resource makes use of a number of types of assessment which share a common theme of communication. The authors have found that the use of CBL resources can be an ideal way of teaching communication skills in a scientific context and it is hoped that this resource will raise awareness of the relevant issues when communicating science to a range of audience types. The following transferable skills are encountered in this problem:

- Working in a small group on a mini-project
- Critical thinking, decision making and independent learning
- Preparing concise written critiques of active areas of scientific research
- Working within a group to critically evaluate a number of different courses of action and justifying the decision made in a short written report
- Writing a short presentation
- Orally communicate an understanding of an area of scientific research

An overview of the transferable skills development in each session is presented in Table 2.

An overview of the scenarios

The scenario puts participants in the role of placement year students working in a food analysis laboratory in the fictional nation of Northland.

Students start by researching topics and sharing findings with other groups of participating students. The problem then moves on to give students the opportunity to consider some experimental data and to analyse it in the context of a realistic problem. The final parts of the problem are more open-ended in nature and reflect the increase in responsibility that students may get as they work through their placements.

Table 2: A breakdown of transferable skills development in each session Transferable skills Week Session Independent learning – auditing existing knowledge and setting research targetsTeam working & group 1 0 (Optional - 60 mins) discussion - considering researched concepts and planning how to use this research to address the problem Communicating scientific concepts – designing a wiki, creating a logical structure and considering how best to connect areas of related content 1(60 - 90 mins)Team working & time management – determining how to work most efficiently by diving a complex task 2 Research skills - performing focused literature searches and web searches Communication skills – preparing a short oral presentation and a written summary 3 2(90 - 120 mins)Communication skills – verbally communicating scientific ideas to an audience of peers and responding to a range of questions on the ideas presented Communication skills - communicating findings in the form of a written report to a specified audience type Planning – formulating appropriate guestions based on a concept presented by a group of peers 3 (90 – 120 mins) Research skills - performing focused literature searches and web searches 4 Data analysis – interpreting experimental data and formulation of conclusions Team working and group discussion - considering researched concepts and planning how to use this research 4 (60 mins) 5 to address the problem Independent learning and critical thinking – applying new knowledge to the problem solution Written communication – preparing a written report for a specified audience type IT skills – using a spreadsheet model 5 (60 mins & 4 – 8 Team working – collaborating on the formulation of an experimental plan 6 hours of lab time) Research skills – performing focused literature searches and web searches Decision making – critically evaluating the literature and deciding which established approaches are relevant to

Week	Session	Transferable skills		
		this problem whilst considering the needs of stakeholders		
		Safety – conducting a safe lab investigation		
7	6 (60 – 90 mins)	Communication skills – preparing an oral presentation		
		Research skills – performing focused literature searches on current research themes		
		Business skills – recognising how the requirements of a for-profit business will influence the viability of various research approaches in an industrial setting		
8	7 (15 mins per group)	Communication skills – verbally communicating scientific ideas to an audience of senior scientists and to respond to a range of questions on the ideas presented		

Resource trials

Some components of this resource were trialled with undergraduate students at the University of Leicester during the resource development stage. The most significant component of the trial was the development of unit 2 which took the form of a student-staff partnership. Students performed the investigation and wrote reports on their findings – some key points from their reports have been included in the facilitator text.

Tutor text

The italic text in this version of the guide is meant to be seen by the tutor only. This text includes guidance on how the problem can be run, marking criteria, feedback from the trials and some (where appropriate) example answers.

Pre-session resources have been provided (eg emails, news stories and suggested reading) for most sessions. You may wish to release these separately in advance of each session. The pre-session resources have been included as appendix 1 in the student version of the document (which you may choose to remove).

Student outputs

'Chemistry and Food Security' is a group-based exercise. By the end of the module student groups should have produced the following outputs:

- A wiki on the structure and function of proteins and DNA (optional preliminary activity run at tutor's discretion).
- A 10 minute group presentation and a one A4 page executive summary on one of the key analytical techniques used in the analysis of meat fraud cases.
- An group report on the outcomes of a food analysis investigation which includes recommendations for immediate action plus recommendations for future work.
- A plan for a simple lab investigation into the adulteration of vodka.
- A plan for laboratory investigation to develop a simple protocol to distinguish between Arabica and Robusta coffee beans.
- A laboratory report outlining the strengths and weaknesses of their coffee analysis approach and outlining their formal recommendations for whether this approach should be adopted.
- A short 'elevator' pitch on a new method to extract and analyse chemical components (including contaminants) from food products.

Suggested timetable

A suggested timetable is shown in Table 3. The resource has been designed with flexibility in mind so you can modify this timetable in order to make the module fit the learning requirements of your own students.

Table 3: A suggested timetable for this module

Week	Session	Topics	Assessment	Pre-session prep/feedback
1	0 (Optional – 60 mins)	Nucleic acids and DNA	Each group will produce a wiki based on the	Before session:
	– 00 mins)	Amino acids	research performed for this part of the problem The 'Se	Issue students with the following:
		Protein structure and function		this part of the problem
		Wikis		The Introductory email from Dave Ball
				In session:
				Start the session by getting students to audit what they already know – this can be done in the context of the Discussion Questions – students can research the discussion questions before the session to help make the in-session group discussion more productive
				Towards the end of the session ask groups to describe the planned structure of their wiki and what research that remains to be done
				After the session:
				Students should receive written feedback (and oral feedback if possible) on their wiki
2	1 (60 – 90 mins)	Background to food adulteration	This session will be used to prepare groups for the	Before session:
		Introduction to protein and	presentations they will give in the next session	Issue students with the following:
		DNA analysis		The 'Session 1' summary which includes the ILOs and the Discussion Questions – you may want to remind students that

Week	Session	Topics	Assessment	Pre-session prep/feedback
		Liquid chromatography- mass spectrometry (LC- MS)		 they can research the discussion questions before the session to help make the in-session group discussion more productive The Introductory email from Dave Ball In session: Start the session by getting students to audit what they already know – this can be done in the context of the Discussion Questions Later in the session encourage students to plan their presentation – what points will they need to make? What additional research needs to be done? Who will do what? Will the group practice the presentation? After the session: Remind students of the deadline (next week) for their written summary and the fact that they will be delivering their presentations in the next session
3	2 (90 – 120 mins)	Background to food adulteration Introduction to protein and DNA analysis Liquid chromatography- mass spectrometry (LC- MS)	This session will be used for the group presentations	 Before session: You should create a schedule for the presentations based on the number of groups you have Issue students with the following: The 'Session 2' summary which includes the ILOs. In session: You may want to get students to peer mark each other based on the assessment criteria at the end of the document

Week	Session	Topics	Assessment	Pre-session prep/feedback
4	3 (90 – 120 mins)	Real-time PCR Analysis of results of DNA analysis experiments Data analysis and forming conclusions	Groups will prepare a short report including an analysis of the data provided by Dave Ball	Before session: Issue students with the following: The 'session 3' summary which includes the ILOs and Discussion Questions. The newspaper stories and the correspondence from Dave Ball (including the data) The template for the report In session: Start the session by getting students to audit what they already know – this can be done in the context of the Discussion Questions Later in the session students need to analyse the data they have been provided – you may want to do this in a room with computer facilities so students can start working on a calibration plot – if this is not possible, they can work on this after the session Before next session: Students need to submit their short report on the data they have been provided with
5	4 (60 mins)	Introduction to the chemical analysis of drinks	Groups will develop an experimental investigation based on established protocols. Groups should define which variables they will test.	Before session: Issue students with the following: The email from Dave Ball on vodka analysis The 'session 4' summary which includes the ILOs. In session:

Week	Session	Topics	Assessment	Pre-session prep/feedback
6	5 (60 mins & 4 – 8 hours of lab time)	The adulteration of coffee products Experimental approaches used to detect food fraud in coffee production	Groups must prepare laboratory plans which will be checked before lab sessions After the lab sessions, students will submit a full report of their investigation	During the session ask groups to describe their approach to you and give some oral feedback on this. Before next session: Students receive written formative feedback their plan (before the next session if possible) Before session: Issue students with the following: The email from Dave Ball The 'session 5' summary which includes the ILOs In session: During the session ask groups to describe their approach to you and give some oral feedback on this After the session: Get students to submit their lab plans and give them feedback before the lab sessions if possible
7	6 (60 – 90 mins)	Introduction to molecularly imprinted polymers Applications of molecularly imprinted polymers	Groups will prepare an 'elevator pitch' based on their research on the detection and extraction of contaminants and toxins in food products	Before session: Issue students with the following: The email from Dave Ball The 'session 6' summary which includes the ILOs In session: During the session ask groups to describe their pitch with you and provide feedback

Week	Session	Topics	Assessment	Pre-session prep/feedback
8	7 (15 mins per group)	Introduction to molecularly imprinted polymers Applications of molecularly imprinted polymers	This session will be used for the group elevator pitch presentations	After the session:Students need to finish preparation of their elevator pitchStudents should be advised to practice their pitch in front of an audienceBefore session:You should create a schedule for the presentations based on the number of groups you haveIssue students with the following:The 'session 7' summary which includes the ILOs After the session:Provide students with feedback on their presentations

Student introduction

'Chemistry and Food Security' is a group case study which will guide you through some of the applications of chemistry in the food industry. The problem will include an introduction to analytical and bioanalytical approaches to detecting food fraud as well as the use of new materials to assist in the detection and extraction of specific chemical species (eg contaminants) from food.

Emails and news stories

Throughout this problem you will be presented with a number of messages in the form of emails. These emails include important information on what you need to do for each part of the problem. Read them carefully and in your groups decide how best to respond.

You will also see a number of news stories relevant to the problem. These stories will provide some additional background information to the problem and will also contain some information that you will need to consider when preparing your solutions to the problem.

Learning outcomes and pre-session preparation

The resource includes a list of relevant intended learning outcomes from each session. This acts as a check list for what you should be able to do after tackling the part of the problem covered in that session. The assessments for each part of the problem is aligned to these lists so please make sure you demonstrate the competencies listed in your assessed work.

The pre-session preparation should guide your research before each session. It is worth remembering that the information presented in the problem is meant to be a starting point, you will need to do further research to fully prepare for each session.

Assessment

This resource makes use of a range of different types of assessment based on the general theme of science communication in the workplace. Communicating your understanding to a range of different audience types in a number of different ways is a very important skill to have. This resource aims to give you the opportunity to develop a range of communication skills and to make key decisions based on your scientific understanding of various concepts combined with an understanding of related political and business factors.

Facilitation

You will be guided through the problem solving process by a facilitator (or tutor). Although your facilitator can provide advice on problem solving strategies, the facilitator will not freely give information about the problem away. Your facilitator will help you by encouraging discussion amongst the group and (if needed) focussing this discussion.

The scenario

This module aims to provide you with a learning experience which familiarises you with a key area of industrial chemical research, food analysis. The problems place you in the role of an undergraduate chemist who is working on an industrial placement for a food analysis laboratory in Northchester, the capital city of the fictional nation of Northland (which you may assume is in the European Union and is very near to the UK). The food legislation of Northland parallels that of the UK – any differences in policy will be highlighted in the problem text. You will work as part of a team of placement students on a number of problems which are based on the science which underpins the quality assurance of food products and will give you some experience of how to communicate findings to a range of audiences.

Welcome email

Dear Placement Students,

I want to welcome you to your new role as undergraduate placement students at Northland Food Analysis Laboratories Ltd. During your time here you will be given the opportunity to apply a number of different approaches used to detect food fraud and isolate contaminants from food products.

Best wishes, Sarah Robinson

Manager of Biochemical Analysis Laboratory Northland Food Analysis Laboratories Ltd Unit 1: Investigation of food fraud in meat products

Pre-session 0 preparation (for the tutor)

Note: this is an optional preliminary workshop that is based on the structure and properties of DNA and proteins. If your students already have a strong background in this area, you may find it more appropriate to start with session 1.

You need to make the following resource available to students before the session:

Email

Dear Placement Students,

It's a pleasure to welcome you to Northland Food Analysis Laboratories. I will be supervising all of our placement students this year. During this placement I want you to work in close cooperation with our permanent staff and the other placement students. You will be involved in the investigation of cases of potential food fraud. Due to client confidentiality, I cannot tell you any more about the project at this stage.

In order to prepare you for this project, I would like you to review what you know about proteins and DNA by creating a small wiki on the staff intranet which provides a scientific background on these important concepts. You can decide on the structure and number of pages in the wiki but you should ensure that the wiki provides enough background to teach someone in year one of a chemistry degree (who hasn't done A-level biology) about proteins and DNA.

Best wishes, Dave Ball

Senior analyst, Northland Food Analysis Laboratories Ltd.

Note: You can either get each individual group to create their own wiki or you can get the whole class to work on a single wiki (perhaps each group can start by creating a single page and then they can review the pages created by other groups). Remember to encourage groups to make effective use of hyperlinks to connect related pages.

You may choose to host the wiki on your virtual learning environment (we have used Blackboard for wiki based CBL and PBL (problem based learning) activities since 2007), If your VLE does not support wikis there are a number of other wiki providers available (eg www.pbworks.com).

For further guidance, see section 2.2 of the following article:

- Seery, M., "Technology Enhanced Learning In The Chemistry Classroom", ICTT Conference, 2013, <u>http://bit.ly/225pm1V</u>
- Buckland, R., "Wikis in University Teaching and Learning", YouTube/UNSW, <u>http://bit.ly/1JhJV25</u> (accessed 22/04/2015)

Session 0 (60 minutes)

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

• The structure and function of proteins and nucleic acids.

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

- Describe how proteins are formed from amino acids and how the functionality of the constituent amino acids affect the properties of proteins
- Describe the structure and role of nucleic acids

TRANSFERABLE

By the end of this part of the problem students should be able to:

- Reflect on elements of previous learning and apply them to a new context
- Work as part of a small team to develop an understanding of key scientific concepts
- Design wiki pages to communicate group findings

Resources and arrangements

• This session will work best if students have access to computer facilities (eg tablet devices, laptops or PCs) with internet access which will allow them to conduct research as they tackle the problem.

Discussion questions

NUCLEIC ACIDS AND DNA

What is a helix? What is a double helix?

A helix is a repeating spiral pattern in the structure of a macromolecule. A double helix consists of two matching helices which are intertwined around a common axis.

What makes up the backbone of DNA?

DNA is composed of pentose sugar (deoxyribose) and phosphate backbone

How are the two strands of DNA connected together?

The two strands are connected by hydrogen bonds between the nitrogen bases in each strand.

What are base pairs and how are they connected to the rest of the DNA molecule?

The bases consist of a group of purine and pyrimidine derivatives. In DNA the purines are adenine and guanine; the pyrimidines are cytosine and thymine. The bases are connected to deoxyribose to form nucleosides, the phosphate monoesters of which are nucleotides. Base pairs are formed by the hydrogen bonds formed between two bases on the two strands of the double helix. Only certain combinations of bases can form base pairs: adenine (A) forms a base pair with thymine (T) and guanine (G) forms a base pair with cytosine (C).

What are the differences between DNA and RNA?

Both are nucleic acids. The primary structure of RNA is essentially the same as that of DNA but the sugar component is different (ribose in RNA, deoxyribose in DNA). There is also a difference in the four bases that appear in the two types of structure: DNA structures incorporate one of the following four bases: adenine, guanine, thymine and cytosine. In RNA uracil replaces thymine.

RNA is not a double helix but there are regions of helical secondary structure. In terms of the biological function of RNA, students should discuss messenger RNA, transfer RNA and ribosomal RNA and their roles in protein synthesis. DNA includes the genetic code for proteins bit it is unable to create the proteins directly (this is done by RNA).

Groups may wish to discuss the formation of messenger RNA from the unravelled DNA template.

How long are typical strands of DNA?

There are 247 million nucleotide base pairs in chromosome 1 – the largest human chromosome.

What are the functions of nucleic acids in cells?

Functions of DNA include the storage of genetic information, the synthesis of RNA and protein synthesis (via the base sequence). Functions of RNA include the direction of protein synthesis and transmission of genetic information. ATP acts as a short term energy store.

What happens to nucleic acids after they are eaten?

Nucleic acids are broken down in the small intestine by nucleases.

Why aren't nucleic acids or nucleotides essential in the diet?

The body can biosynthesise nucleotides and purine and pyrimidine bases.

AMINO ACIDS AND PROTEIN STRUCTURE AND FUNCTION

What happens to protein when we eat it?

Students should discuss the digestion of proteins (in the stomach and duodenum) into free amino acids (by HCI which separates proteins into amino acids followed by enzyme catalysed digestion of the amino acids) which are then metabolised.

Why do we need protein in the diet?

Proteins act as sources of amino acids (including essential amino acids – those which cannot be biosynthesised by the human body) which are used to biosynthesise the proteins essential to life.

What are typical functions of proteins in the body?

Proteins have a wide range of functions in the body. These include: Structural support, storage of amino acids, transport of other proteins and molecules, movement, communication between and within cells etc. There are a large number of functions – students should aim to list these and explain how a single type of molecule can perform such a diverse range of roles.

What chemical bonds are present in proteins?

Peptide (C–N) bonds between successive amino acids in the protein chain are of key importance. Students should know the general condensation reaction scheme too. Weaker forces and bonds between different parts of the protein are discussed in questions below.

What are the four levels of protein structure and their significance?

Primary – the amino acid sequence. These amino acid residues are held together by covalent peptide bonds. Secondary – the highly regular local sub-structure (ie. A–helix and β –sheets structure) – hydrogen bonding plays an important part at this level, Tertiary – the 3D structure of the protein: this level of structure is dominated by weaker intermolecular interactions between groups in different parts of the protein chain which fold the protein into a compact globule, Quarternary – the assembly of several proteins or polypeptides.

What is an essential amino acid?

This is an amino acid which cannot be biosynthesised by the body (see second question).

What is the relationship between amino acids and proteins?

Proteins are effectively long chains of amino acids held together by peptide (C–N) bonds. The actual structure is more complicated due to a range of other weak physical and strong chemical interactions between different parts of the chain (see structure question above).

What factors influence the final structure of a protein?

A number of strong chemical and weak physical interactions including hydrogen bonds between amine and carbonyl groups of amino acids within the protein backbone, ionic bonds between oppositely charged groups within the structure, hydrophobic effects – the tendency of non-polar groups to cluster together to form micelles, van der Waals interactions between non-polar groups (students can discuss the range of interaction types possible within proteins) and disulfide bridges which are covalent links between two cysteine groups.

WIKIS

What is a wiki? Give at least two common examples of wikis you may have used.

Wikis are collaborative web pages which allow a group of people to work together to create and develop content. Wikis are easily editable and usually have a comments feature which allow collaborators to comment on the structure and direction that the page is taking (the comments feature can alkso be used to generate feedback from users). Two common examples are Wikipedia and ChemWiki

(<u>http://chemwiki.ucdavis.edu/</u>) but there are many other examples of specialist wikis that students may be aware of.

How does a wiki differ from a normal web page?

There are several key differences: wiki pages should be collaborative so the user can add additional content, wiki pages tend to be highly hyper-linked in order to highlight areas of related content and individual wiki pages are often very closely aligned to a single topic.

Deliverable

By the next session you will need to submit the following:

• Your group wiki on amino acids and DNA

We suggest you give students until session 1 (typically around 1 week) to prepare their wiki. You can potentially get students to peer assess the wiki pages or run this as a formatively assessed activity as it is essentially a preliminary task to prepare students for the main part of the problem.

Pre-session 1 preparation (for the tutor)

You need to make the following resources available to students before the session:

Food fraud fear in Northland stores Northland Guardian

Northland retailers have been left shocked by news that one of the main producers and distributors of processed meat products in Western Europe has been supplying beef products which may contain significant amounts of horse meat. The contaminated products were detected in products that MP Limited has produced and distributed for sale in an international chain of supermarkets which has branches in France, Belgium and Spain. The same company is one of the major suppliers of meat products to restaurants and supermarkets throughout Northland. The Northland government has demanded an investigation into a range of products to ascertain the extent of any possible fraudulent products which have reached Northland supermarkets. The news has caused concern amongst Northland's consumers and religious leaders...

Email

Dear Placement Students,

I'm sorry to cut short your induction to the company but we urgently need your help. The Northland government has awarded us a contract to investigate the recent food fraud case which has been in the news. As we are currently very busy undertaking the investigation, we need you to bring yourselves up to speed by researching the key components of food fraud investigations. Our investigation is based on three main forms of analysis:

The detection (and potential quantification) of equine (horse) DNA in meat products intended for human consumption

The detection of equine (horse) protein in meat products intended for human consumption

The detection of phenylbutazone (a veterinary drug not suitable for use in humans) in meat products intended for human consumption by liquid chromatography – mass spectrometry (LC-MS)

In the groups you have been assigned to, we would like you to choose one of these approaches to research (you may want to base this decision on your university studies) and to prepare a ten minute group presentation which you will deliver to all of the other placement students working at the company. You also need to prepare a one (A4) page executive summary on your chosen techniques that placement students can refer to. Please include some labelled diagrams and remember to describe these processes in the context of a food fraud investigation. Please be prepared to ask the other groups questions about their presentations as you will need to apply what you learn about all of these techniques very soon.

I have included some details of some useful articles that will help you start your research below.

Best wishes, Dave Ball,

Senior analyst, Northland Food Analysis Laboratories Ltd.

We suggest you give students until session 2 (typically around 1 week) to prepare their presentation and one page summary. You need to get at least one group per topic so you may want to assign topics to groups rather than allowing them to choose.

We have intentionally not stated a format for the presentation as we feel this choice is best made by the tutor. You may impose a standard format (eg all groups must use PowerPoint) or you may allow groups to select the format that they feel is most suitable.

Suggested resources

- Ballin, N. Z., Vogensen, F. K., Karlsson, A. H., "Species Determination Can we detect and quantify meat adulteration?", Meat Science, 2009, 83, 165–174.
- ABC News (Video), "Food Fraud? Watchdog Group Raises Concerns", 2013, <u>http://bit.ly/24UCGoL</u> (accessed 22/04/2015)
- BBC News (Video), "Horsemeat Scandal: Inside Lab Testing Products", 2013, <u>http://bbc.in/1Rb6c5H</u> (accessed 09/04/2015).
- Birch, H., "The Food Detectives", Chemistry World, 2009, 6, 10, 58–62.
- Edith, I. N., Ochubiojo, E. M., "Food Quality Control: History, Present and Future", chapter in "Scientific Health and Social Aspects of the Food Industry" edited by Caldez, B., 2012, Intech
- Hsieh, Y-H. P. and Ofori, J. A., "Detection of Horse Meat Contamination in Raw and Heat-Processed Meat Products", J. Agric. Food Chem., 2014, 62, 12536– 12544.
- Perks, B., "Fighting Food Fraud with Science", Chemistry World, 2014, 48–52

• Walker, M.J, Burns, M., Burns, D., T. "Horse Meat in Beef Products-Species Substitution 2013", Journal of the Association of Public Analysts, 2013, 41, 67–106.

Session 1 (60 – 90 minutes)

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

 Food adulteration (both accidental and intentional), qualitative and quantitative testing and analytical and bioanalytical techniques used to detect food adulteration including DNA analysis by Polymerase Chain Reaction (PCR), protein analysis by Enzyme-linked Immunosorbent Assay (ELISA) and trace chemical analysis liquid chromatography-mass spectrometry (LC-MS).

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

- Recognise that food adulteration (intentional and accidental) has been an issue throughout time
- Discuss the legal measures that have been put in place to help combat food adulteration
- Describe the scientific basis of the techniques used to detect meat adulteration and the relative merits of these techniques to specific cases
- Evaluate the potential risk to human health from contaminants in adulterated meat supplies

TRANSFERABLE

By the end of this part of the problem students should be able to:

- Work in a team to research a range of analytical methods used in food analysis
- Perform literature searches on an active area of research in order to gain a greater understanding of how fundamental scientific concepts are applied to current research
- Work in groups to produce written summaries of scientific research suitable for a range of different audiences and present the key findings in a short talk

Resources and arrangements

• This session will work best if students have access to computer facilities (eg tablet devices, laptops or PCs) with internet access which will allow them to conduct research as they tackle the problem.

Discussion questions

BACKGROUND TO FOOD ADULTERATION (FOOD FRAUD)

What is meant by food adulteration? Why might food be adulterated?

Food adulteration may be intentional (i.e. someone has added another ingredient to reduce the cost of production and to make a product which appears to have a higher value than it actually does) or accidental (possibly due to a failure in quality control procedures). Prior to the introduction of legislature, it was common for food manufacturers to add a variety of toxic ingredients to improve the taste, appearance or weight of a product (eg adding alum and chalk to flour when making bread and adding strychnine to enhance the taste of beer).

What legislation exists to protect consumers from food adulteration?

The first food law in England was introduced by King John to prevent the adulteration of bread.

In the late 18th century and early 19th century the work of an analytical chemist (Accum) and a medic (Hassall) exposed the extent of food adulteration in 19th century Britain. This led to the Food Adulteration act of 1860, the first modern law enacted to protect consumers from food adulteration.

The interests of British consumers are now protected by the UK Food Safety Act 1990, Regulation (EC)178/2002 of the European Parliament and the Codex Alimentarius (2003) of the World Health Organization.

Safety checks can be carried out throughout the food supply chain. What are the four key components of this supply chain?

- Source of raw ingredients
- Site of production
- Processing sites and distribution
- Points of final service (i.e. retailers)

Qualitative tests can be used to identify whether a given contaminant is present or not in a product. Quantitative tests can be used to determine the amount of a contaminant in a sample. Why is qualitative testing still used if the level of detail given in the results is lower than that obtained from quantitative testing?

Qualitative testing can be used to rapidly identify whether a contaminant is present without having to spend as much time (or money) as is needed to run a quantitative test. Quantitative testing can be used as a follow up to positive qualitative tests.

As a group, identify the key stakeholders in cases of food-fraud and briefly discuss the interests of each of these stakeholders.

Consumers – risk of losing out financially by being 'tricked' into paying more for a lower quality product. This may also conflict with religious beliefs and dietary preferences.

Consumers – possibility of eating a substandard product with the associated health risks (including contamination of food supplied by agrochemicals and drugs).

Retailers (to consumers) – Risk legal/civil action being taken against them and negative media attention/public attitude (impact on sales). Financial impact of paying a premium for a low quality product.

Producers/wholesale retailers – Risk legal/civil action being taken against them and negative media attention (impact on sales).

Farmers/landowners – financially penalised by producers.

INTRODUCTION TO DNA AND PROTEIN ANALYSIS

DNA analysis by polymerase chain reaction (PCR) is one of the most commonly used approaches for detecting food fraud. As a group discuss how PCR works and consider how it may be applied to food fraud cases. Highlight any potential difficulties of using this approach.

DNA analysis by polymerase chain reaction (PCR) is one of the leading analytical approaches used to verify (and quantify) the nature of meat used in food and feedstock products. PCR works by amplifying (i.e. making a large number of copies of) a DNA fragment of interest. The process starts by denaturing DNA at high temperatures (this melts the double helix to leave two single strands) and two different oligonucleotide primers are used to flank the region of interest. The primers are then elongated by deoxynucleotides in the presence of a heat resistant polymerase enzyme (Taq polymerase) at lower temperatures. The process is then repeated which facilitates a chain reaction. A number of techniques can be used to analyse the outcome of a PCR experiment including gel electrophoresis.

PCR analysis may be facilitated by the use of real-time analysis (eg fluorogenic probes). Probes can be designed to be highly selective for specific nucleotide sequences thus providing a fast, cheap and simple means of detecting given DNA sequences.

PCR analysis can be difficult to interpret as a w/w contamination value. This is because of the use of mitochondrial DNA. The amount of mitochondrial DNA varies in different tissues so correlation of the PCR result will be wrong unless the tissue composition is known (not normally the case).

Analysis of cases of potential meat fraud may also make use of protein analysis by Enzyme-linked immunosorbent assay (ELISA). As a group discuss how ELISA works and consider how it may be applied to food fraud cases. Protein analysis by Enzyme-linked immunosorbent assay (ELISA) is another leading analytical technique which is fast, relatively cheap, provides a simple colour-change based result and can be used in the field. ELISA is based on the detection of specific antigens. This is achieved by attaching the antibody for the protein (antigen) of interest to a polymer support. The sample (in the form of a drop of cell extract, serum or urine) is then laid on the sheet. After the formation of the antigen-antibody complex, the sheet is washed to remove any unbound sample. The final stages involve the addition of second antibody which is attached to an enzyme. The enzyme attaches to a different site on the analyte protein. An enzyme is chosen which will produce an intensely coloured complex or a fluorescent complex.

Compare and contrast the use of ELISA and PCR in these kinds of analyses and consider the relative advantages of the different approaches.

DNA has a higher thermal stability than proteins; it is present in the majority of cells and potentially enables identical information to be obtained from different samples of the same animal.

Briefly discuss any other techniques that may be used in the analysis of meat fraud and consider the relative merits of these approaches.

Liquid chromatographic and capillary gel electrophoretic methods have been used in the qualitative detection of proteins in meat products.

DNA hybridisation is an inexpensive analytical process but most applications only yield qualitative data. Although there has been some demonstration of quantitative analysis, applications are very limited. DNA hybridisation is less sensitive than PCR.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

LC-MS can be used to help detect (and quantify) phenylbutazone present in samples of meat. Briefly describe the operating principle of LC-MS.

LC-MS approaches usually use HPLC instrumentation. This involves forcing a liquid mobile phase through a column which has been packed with a suitable stationary phase. This approach facilitates the separation of the components of a liquid mixture. Mass spectrometry can be used to analyse the different components.

What is the chemical structure of phenylbutazone?



Phenylbutazone

Figure 1: The structure of phenylbutazone

What are the primary uses of phenylbutazone?

Phenylbutazone is used as an anti-inflammatory drug in horses. Historically it was used to treat humans but this is no longer the case (see the last question of this section for an explanation of why it has been banned).

What measures are put in place to prevent meat from horses exposed to phenylbutazone from entering the human food chain in the EU?

Horses that are treated with phenylbutazone have this recorded on their Food Chain Information (FCI) forms. Horses with this record on their FCI forms are permanently excluded from the human food chain.

What are the risks posed to human health by phenylbutazone?

Phenylbutazone was previously prescribed as a treatment for gout and rheumatoid arthritis (Walker, et al., 2013). The subsequent discovery that the drug can be fatal to humans has resulted in it being withdrawn from use (apart from in a small number of very specific cases).

Deliverable

By the next session each group needs to have prepared the following:

- The group presentation which you will deliver (you should also be prepared to answer questions on your research)
- The one page executive summary of your group research

Session 2 (90 – 120 minutes)

This session will be used for the group presentations. It can be useful to get students to peer review each other's presentations (see outline marking criteria). We suggest dividing this session up into a series of three presentation long sub-sessions. Each presentation will take around 15 minutes (including around 5 minutes for questions) so each of the three sub-sessions will take around 45 minutes. It would make sense to divide the groups up so that each sub-session consists of just a single talk on each of the three analytical approaches.

The questions at the end of each talk should be student led but you may need to ask one or two seed questions. When thinking of potential questions, you should encourage students to reflect on the group discussion from the last session.

You need to make sure this room has a suitable capacity and has any audio-visual (and/or IT) support that students may need when giving their presentations and answering questions.

Feedback from the tutor should focus on the quality of the delivery of the presentation (and answers to questions) as well as the level of research that the group had done before the session. You may find it useful to base your feedback on the marking criteria given at the end of this document.

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

- Your chosen approach (from session 1) so that you can participate in a short group presentation on this topic and answer questions from your peers.
- The other analytical approaches discussed in the last session so you can ask your peers questions about their presentations.

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

• Describe the scientific background of an analytical approach used to investigate food fraud and evaluate the relative strengths and weaknesses of this approach in the context of the other available approaches.

TRANSFERABLE

By the end of this part of the problem students should be able to:

• Verbally communicate scientific ideas to an audience of peers and to respond to a range of questions on the ideas presented

Formulate appropriate questions based on a concept presented by a group of peers

Deliverable

• In this session you will deliver your group presentation

Pre-session 3 preparation (for the tutor)

You need to make the following resources available to students before the session:

Adulterated meat could be harmful to your health warns minister Northland Guardian

Northland minister for health Jeff Gayle has warned that meat products contaminated with horse meat may contain a veterinary drug known as phenylbutazone (or 'bute'). The drug is routinely used as a pain killer in horses but is excluded from the human food chain in Northland. Phenylbutazone is known to cause a number of disorders in humans including potentially fatal liver damage. Mr Gayle stated that there is no evidence to indicate that phenylbutazone has entered the human food chain but ongoing tests will attempt to ascertain whether the drug has been present in meat products sold in Northland.

Contaminated meat may contain lethal chemicals Northchester Gazette

There are fears that a recent outbreak of illness in Northchester may be related to the possible contamination of meat products that have been sold throughout Northland. It is possible that meat products which may contain alarming amounts of horse DNA may also be contaminated by a range of drugs which are not suitable for entry into the human food chain. An anonymous source from the medical profession told the Gazette that there have been a number of severe cases of illness in Northland which may be consistent with symptoms of poisoning by the illegal drug phenylbutazone (known to vets as 'bute').

Email

Dear Placement Students,

I'm afraid the food fraud situation has escalated as you will have probably already seen in the news. There is now concern that contaminated meat products may have introduced drugs that are not fit for human consumption into the human food chain. I have attached some data from our ongoing analysis. I have asked everyone to take a look at this data and to write an analysis of the results as I believe everyone will bring something different to this analysis.

Please use the attached template to help you write a short report on the results. Essentially we need to know the following:

What concentrations of contaminant (in ng/µI) are present in these samples according to the results? What are the w/w percentages of equine DNA relative to total DNA content?

What are the limitations of these results? What conclusions can we draw in terms of what we report back to the government of Northland?

Can we use these results to state whether any of these products constitute cases of gross adulteration?

Is there a realistic risk to the population of Northland from phenylbutazone contamination of consumer food products?

Northland's food legislation defines gross adulteration as being characterised by 1% (weight of contaminant/weight of total meat in product) or more of the meat content of the food product being horse meat.

Best wishes, Dave Ball

Senior analyst Northland Food Analysis Laboratories Ltd

Data

Table 4: DNA analysis data

Sample	Total DNA concentration (ng / μl)	Phenylbutazone (LC-MS)	Cq value	Comments
NCFL01	74.12	Negative	N/A	SmartSaver frozen lasagne (Sample 1)
NCFL02	72.22	Negative	13.007	SmartSaver frozen lasagne (Sample 2)
NCFB01	65.12	Negative	N/A	Fast Burger quarter pounder (Sample 1)
NCFB02	64.08	Negative	N/A	Fast Burger quarter pounder (Sample 2)
NCBS01	68.51	Negative	N/A	SmartSaver beef sausage (Sample 2)
NCBS02	70.03	Negative	N/A	SmartSaver beef sausage (Sample 2)
NCAB01*	71.12	Negative	13.759	Northchester army barracks canned meat reserve (Sample 1)
NCAB02*	73.25	Positive	13.858	Northchester army barracks canned meat reserve (sample 2)

The real-time PCR instrument used in this experiment has a limit of detection (LOD) of 0.1%.

The analysis was run for mitochondrial horse DNA.

Results were obtained by adding a fluorescent probe to the DNA mix. The cycle number (Cq) that a threshold value of fluorescence was detected has been recorded.

*Note – samples NCAB01 and NCAB02 also showed higher than normal levels of some trace metal species including tin and lead.
Real-time PCR calibration data

Cq value	Equine DNA concentration (ng / µl)				
10	0.0664				
15	1.0476				
20	37.4111				
25	900.6582				

Table 5: PCR calibration data

Note: the following table and plot are for the tutor's eyes only!

Table 6: DNA analysis data – for tutor only

Sample	Equine DNA concentration (ng / µl)	Total DNA concentration (ng / μl)	Phenylbutazone (LC-MS)	Cq value	Comments
NCFL01	None detected	74.12	Negative	N/A	SmartSaver frozen lasagne (Sample 1)
NCFL02	0.386	72.22	Negative	13.007	SmartSaver frozen lasagne (Sample 2)
NCFB01	None detected	65.12	Negative	N/A	Fast Burger quarter pounder (Sample 1)
NCFB02	None detected	64.08	Negative	N/A	Fast Burger quarter pounder (Sample 2)
NCBS01	None detected	68.51	Negative	N/A	SmartSaver beef sausage (Sample 2)
NCBS02	None detected	70.03	Negative	N/A	SmartSaver beef sausage (Sample 2)
NCAB01	0.626	71.12	Negative	13.759	Northchester army barracks canned meat reserve (Sample 1)
NCAB02	0.667	73.25	Positive	13.858	Northchester army barracks canned meat reserve (sample 2)

Note: the following plot is for the tutor's eyes only.



Figure 2: Calibration plot from RT-PCR of equine mitochondrial DNA

Guidance: Students need to use the Cq values of the known standards to plot a calibration plot (Cq versus negative log concentration). Using the equation of this line, students can calculate the concentrations (in ng/μ) of each of the unknown samples and then determine a w/w percentage of horse meat present in the three samples where equine DNA have been detected. The primary problem with this approach is the fact that mitochondrial DNA has been used so although the students can calculate numerical values, they have to state that these values are very approximate (for the reasons mentioned in the discussion questions). Additionally, calculating a w/w percentage can be misleading due to differences in the in genome size between different animals.

In terms of phenylbutazone contamination, although one sample has tested positive, this sample has been sourced from canned meat reserves at an army barracks. It is possible that these reserves were canned at a time that predates the current legal measures to ensure that horses treated with phenylbutazone did not enter the human food chain (the elevated levels of trace metals suggest long term storage is possible).

It appears that most of the limited range of consumer food products tested are free from phenylbutazone contamination but one product has tested positive for horse DNA. The most sensible course of action would be to immediately remove this product from sale and conduct a more extensive testing programme on a wider range of products to establish the extent of the problem.

Session 3 (90 – 120 minutes)

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

• Food adulteration (both accidental and intentional), qualitative and quantitative testing and analytical and bioanalytical techniques used to detect food adulteration (including DNA analysis by Polymerase Chain Reaction (PCR) and protein analysis by Enzyme-linked Immunosorbent Assay (ELISA)).

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

- Consider the applicability of real time PCR in the analysis of meat products and identify the difficulties in using the data generated by this approach to generate a solution to the problem
- Use data generated by other types of analysis to help inform your conclusions
- Analyse a set of data in order to generate conclusions relevant to the context of a specified problem

TRANSFERABLE

By the end of this part of the problem students should be able to:

- Perform literature searches on an active area of research in order to gain a greater understanding of fundamental scientific concepts are applied to current research
- Communicate findings from a scientific study in the form of a written report which addresses the requirements of supervisors and government agencies

Resources and arrangements

• This session will work best if students have access to computer facilities (eg tablet devices, laptops or PCs) with internet access which will allow them to conduct research as they tackle the problem.

Discussion questions

REAL-TIME PCR

What are the key differences between real-time PCR and the standard PCR approach? What are the advantages of real-time PCR in food analysis?

A fluorescent probe is added to the PCR mix which results in the release of a quenched fluorescence signal which is measured during each PCR cycle. The fluorescence signals are captured by a camera and the data is stored and processed by a computer.

How is the concentration of target DNA quantified by real-time PCR?

Real-time PCR analysis of a number of standard samples (of known concentrations) must be run. Samples with a higher concentration of target DNA will begin to show amplified fluorescence at an earlier PCR cycle. A threshold relative fluorescence is set and the cycle number that each of the standards reach that threshold is recorded (as the Cq number). The Cq number of the standards is then plotted against the negative logarithm of the concentration to create a calibration plot. The unknown samples are then analysed in the same way and the Cq numbers are recorded. The calibration curve is then used to give the concentrations of the unknown samples.

PCR analysis frequently makes use of mitochondrial DNA. What is mitochondrial DNA and why it is frequently used as the basis of this type of analysis?

Mitochondria are components of cells that produce energy. Mitochondria are present in all cells in high numbers meaning they can be detected from tissue from any part of an animal.

How is DNA extracted from meat samples for real time PCR analysis?

Students will find that a number of different approaches exist which usually involved grinding tissue followed by addition of a buffer and an extraction enzyme. Samples are usually vortexed and incubated to maximise extraction. DNA and RNA may then be recovered by centrifugation. Students will be able to find protocols online (The Food Standards Agency has published standard protocols for this).

Why is quantification of a PCR result difficult when mitochondrial DNA is used?

Mitochondrial copy number is highly variable within the tissue of a single animal and from one animal to another so exact quantification is almost impossible. Degradation of DNA during processing and/or cooking can also be an issue. The majority of DNA based analyses are essentially qualitative although some approximate quantification is often attempted. Alternative approaches like real time PCR using single copy genomic DNA allows more reliable quantification as the copy number is constant but this approach has a higher limit of detection.

What is meant by the term Limit of Detection (LOD)?

The Limit of Detection of analytical approach is the lowest concentration of analyte that can be detected by the process.

ANALYSIS OF RESULTS

How can the real time PCR data be converted into quantities of equine DNA? How reliable will the quantification be?

Use data for the standard solutions to prepare a calibration plot. Use the equation of a straight line to determine the concentrations of equine DNA in all samples that tested positive. Determine w/w percentage using the determined amount of equine DNA and the total amount of DNA quoted in the table of results. The quantification will not be particularly reliable as mitochondria DNA has been used for this analysis (see answers to questions in previous section).

What is the significance of the higher than normal detected levels of trace metal ions present in the last two samples?

This suggests metal ions may have leached into the food product from the container. This may be an indication that the food has been stored for an extended period of time which may be of significance when deciding whether the results pose a threat to human health. The presence of lead probably indicates that cans were soldered.

Deliverable

• A report on the results of this analysis using the template provided by Dave Ball.

Unit 2: Investigating adulteration of drinks

Pre-session 4 preparation (for the tutor)

You need to make the following resources available to students before the session:

Email

Dear Placement Students,

We have been contacted by Northchester University who would like us to investigate a case of suspected drink fraud. In the last seven days a group of students have suffered prolonged spells of severe illness. The students have experienced symptoms including severe headaches, blurred vision, dizzy spells and vomiting over a two day period since they became ill. The University's internal investigation appears to trace this back to the Northland Union of Students' 'Double Vision' event (a night where double measures of spirits are sold at the price of single measures) which took place last Tuesday.

Interviews with the affected students suggest that the possible cause was vodka sold at the 'Double Vision' night. We need to investigate samples of vodka collected from the university to establish the nature of the problem. This should be a relatively simple investigation so I thought it would be good experience for you to plan a suitable experiment to measure this which will be run in parallel with our normal investigation. Please design an experimental investigation which you would be able to run using the standard equipment in our laboratory (eg HPLC, UV-Visible absorption spectrometer, infrared spectrometer, etc.) and write a one page plan which outlines how to run the investigation, what equipment and reagents are needed and what the anticipated results would be.

Best wishes, Dave Ball

Senior analyst Northland Food Analysis Laboratories Ltd.

Suggested resources

- Barbosa-García, O., Ramos-Ortíz, G., Maldonado, J. L., Pichardo-Molina, J. L., Meneses-Nava, M. A., Landgrave, J. E. A., Cervantes-Martínez. "UV–vis absorption spectroscopy and multivariate analysis as a method to discriminate tequila", J., Spectrochim. Acta A, 2007, 66, 129.
- BBC News (Video), "Czechs Ban Spirits After Bootleg Alcohol Poisoning", 2012, http://bbc.in/1RDyv71 (Accessed 09/04/2015)
- MacKenzie, W. M.; Aylott, R. I. "Analytical strategies to confirm Scotch whisky authenticity. Part II: Mobile brand authentication", Analyst. 2004,129 (7) :607–12

Session 4 (60 minutes)

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

• The contamination of alcoholic drinks, spectroscopic and chromatographic techniques.

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

• Evaluate a range of experimental approaches used to analyse alcoholic drinks and choose a suitable approach for a given problem.

TRANSFERABLE

By the end of this part of the problem students should be able to:

- Write a brief experimental plan which outlines how to run an analytical investigation and provides details on the anticipated results.
- Plan a simple experimental investigation based on established protocols

Resources and arrangements

• This session will work best if students have access to computer facilities (eg tablet devices, laptops or PCs) with internet access which will allow them to conduct research as they tackle the problem.

Discussion questions

Why is the adulteration of alcoholic spirits a problem? What types of adulterants are commonly used?

Adulteration of spirits can allow low quality products to be sold at higher market prices. The adulteration of spirits by water, ethanol or methanol is a major problem. This adulteration can reduce product quality, lead to loss of tax revenue and can produce a major threat to public health.

Some distillers produce low quality products which aren't necessarily a hazard to health but may be mislabelled as premium products to be sold to the public.

Who are the major stakeholders in cases of adulteration of alcoholic spirits?

Consumers – major health risks – some adulterated spirits can contain levels of methanol (or other contaminants) well above tolerance levels. Consumers also risk of losing out financially by being 'tricked' into paying more for a lower quality product.

Retailers (to consumers) – Risk legal/civil action being taken against them and negative media attention/public attitude (impact on sales). Financial impact of paying a premium for a low quality product.

Distillers/wholesale retailers – Risk legal/civil action being taken against them and negative media attention (impact on sales).

Distillers of premium brands – Potential lack of sales due to lack of public confidence that they are getting the brand they have paid for.

Government – loss of tax revenue.

In this specific case, the University and its student union are also clearly stakeholders as they have a responsibility to ensure the safety of their students. They are also obliged to sell products which conform to food safety law.

Research and discuss the experimental approaches commonly used to detect adulteration of spirits. Discuss the difficulties associated with some of these methods.

Traditionally detected by organoleptic analysis (based on taste, appearance, scent, etc.) but this is a highly subjective method.

Chromatographic approaches been developed. Ion-chromatography, gaschromatography (eg with flame ionisation detectors) and liquid-chromatography have all been employed to isolate the components of a spirit. Some of these approaches (especially those which employ solid phase extraction) are expensive to run and do not provide unambiguous evidence of brand fraud.

Ion chromatography coupled with chemometric analysis of the results can potentially discriminate between different brands of a single type of spirit on the basis of anion and cation composition. This is said to be a simple and effective approach.

A range of spectroscopic approaches have also been employed which are generally cheaper and easier to run than chromatographic based approaches.

Near-infrared spectroscopy (coupled with chemometric data analysis) has been used to verify the type of spirit present and to establish whether adulterants are present or not. This approach has the disadvantage of being relatively expensive. UV-Visible spectroscopy coupled with chemometric approaches has been used for authenticating spirits. This approach is cheaper and simpler than many of the alternatives discussed here. Identification of adulterants can be achieved by comparing absorbance at a number of characteristic bands. Field analysis is possible.

Flow batch analysis is possible which can eliminate manual handling of samples on spectral instruments.

The determination of isotope ratios using ²H–NMR or ¹³C isotope analysis can distinguish between different brands, they are expensive and time-consuming (eg the analysis must take place in labs).

What simple experimental approaches could be used to identify the presence of methanol in vodka?

Students will find a range of spectroscopic and chromatographic approaches which can be used to distinguish between pure and adulterated vodka products.

Deliverable Before the next session you need to prepare:

• A short plan (maximum one page) that outlines an experimental approach that could be used to identify adulterated samples of vodka. This should include a stepby-step set of instructions on how to run the experiment, a list of reagents, an equipment list and some comments on the expected results.

Note: you may wish to run this as a formative task before the next session which will require the students to develop a full experimental plan which they will use as the basis of a lab-based investigation they will conduct.

Pre-session 5 preparation (for the tutor)

You need to make the following resources available to students before the session:

Email

Dear Placement Students,

The detection of coffee adulteration is a significant challenge. Adulteration of coffee beans affects farmers, processors and suppliers worldwide. Coffee production is dominated by two species of beans: "Arabica" and "Robusta". Arabica is associated with better quality products and is therefore sold at a higher price. "Green", unroasted coffee may be adulterated by producers with the addition of husks and Robusta beans.

We use a range of approaches to detect substitution of Arabica beans by Robusta beans including NMR and PCR but we would like to develop a relatively simple technique which can be used by our partner laboratories around the world which may not have access to the same level of analytical equipment that we have.

I would like you to plan a laboratory investigation into methods that could be used to distinguish between Arabica and Robusta and then to conduct the investigation. You might want to start by attempting a simple caffeine extraction using organic solvents and then determining the quantity of caffeine extracted per unit mass of bean.

I'm not sure what form this investigation will take but I have included a few experimental parameters that you may wish to investigate:

What are the best conditions for the extraction: which solvent works best? Are acidic or basic conditions better?

What approaches can be used to measure caffeine levels after the extraction? Can UV-Vis be used? (maybe you could measure the absorption of some standards to produce a calibration plot?).

Can sublimation be used to help establish caffeine levels?

We need to investigate whether these approaches are reproducible.

You should coordinate your efforts with the other groups in order to broaden the scope of your investigation. The end product should be a group report on your investigation which includes a summary of your key findings and a recommendation of whether the investigated approach should be adopted.

Best wishes, Dave Ball

Senior analyst Northland Food Analysis Laboratories Ltd.

Suggested resources

- Domingues, D. S., Pauli, E. D., de Abreu, J. E., Massura, F.W., Cristiano, V., Santos. M. J., Nixdorf, S. L., "Detection of roasted and ground coffee adulteration by HPLC and by amperometric and by post-column derivatization UV-Vis detection", Food Chem. 2014 Mar 1;146:353–62.
- Royal Society of Chemistry (Video), "High Performance Liquid Chromatography HPLC", 2008, <u>http://bit.ly/225pyOS</u> (accessed 22/04/2015)

This investigation was undertaken by a team of BSc project students at the University of Leicester as part of a student-staff partnership. The Leicester students have investigated ground Arabica and Robusta coffee and have generally found that Robusta beans have higher caffeine content (the cheaper product has more kick to it!) but they have had some difficulty in generating reproducible results. For the extraction, the Leicester students found that DCM was the best solvent and basic conditions worked best. This trial showed that both UV-Vis. and HPLC indicates that Robusta has a higher caffeine content but UV-Vis. suggests higher caffeine levels than HPLC (possibly due to additional DCM-soluble UV active compounds being present). The main limitation of this approach is the fact that it cannot be used to test a single sample to give a definitive result of whether the sample consists of Arabica, Robusta or a mixture).

This planning session needs to be followed by around 8–12 hours of laboratory investigation. If the groups coordinate their efforts, they can explore the widest possible range of different methods and conditions. You may want to place limits on what facilities will be available to students to conduct this investigation. You also need to tell students what safety information they will need to provide (eg COSHH and/or risk assessments) based on your laboratory safety regulations.

A template has been provided for the report but you may want to be flexible in terms of the format of the report.

Session 5 + lab sessions (60 minutes planning session and 8 – 12 hours laboratory investigation)

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

• Adulteration of coffee and the development of an experimental plan to analyse samples of coffee and distinguish between Arabica and Robusta beans.

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

- Consider the methods commonly used to distinguish between Arabica and Robusta coffee beans
- Develop an experimental plan to create a simple method to distinguish between Arabica and Robusta coffee beans.
- Perform a laboratory investigation to measure the quantities of caffeine extracted from ground Arabica and Robusta coffee beans and to analyse the products using a range of different analytical approaches.

TRANSFERABLE

By the end of this part of the problem students should be able to:

- Work in a team to develop an experimental approach based on an open ended problem
- Research a range of experimental approaches and decide which approaches provide potential solutions to a given problem.
- Consider the information required by third-parties from this investigation (eg supervisors, other analysts, government agencies, etc.)
- Closely coordinate a laboratory investigation with a group of collaborators.

Resources and arrangements

- The planning session will work best if students have access to computer facilities (eg tablet devices, laptops or PCs) with internet access which will allow them to conduct research as they tackle the problem.
- The laboratory session will have to be run in a suitable chemistry laboratory. Students will need to prepare the appropriate COSHH/risk assessment statements for their investigations.

Make the following resources available to students before this facilitation session:

Discussion questions

Identify the potential stakeholders in this problem (i.e. coffee adulteration) and briefly describe the nature of their interests.

Consumers – risk of losing out financially by being 'tricked' into paying more for a lower quality product.

Consumers – possibility of drinking genetically modified material.

Government agencies/analytical labs – requirement to develop processes which allow simple, inexpensive determination of adulteration of coffee products.

Retailers (to consumers) – Risk legal/civil action being taken against them and negative media attention/public attitude (impact on sales). Financial impact of paying a premium for a low quality product.

Producers/wholesale retailers – Risk legal/civil action being taken against them and negative media attention (impact on sales).

Farmers/landowners – financially penalised by producers.

Describe some of the approaches that have been developed to detect this type of adulteration.

Chromatographic and spectroscopic approaches can be used to reveal the presence of alien species. The presence of D-5-avenasterol is key evidence for the presence of Robusta material.

Current approaches are based on optical microscopic analysis of consumer products samples at random. Expensive (requires a lot of expertise) and lengthy process.

The detection of DNA molecular markers can be used to discriminate between Arabica and Robusta and even among different Arabica varieties.

PCR-amplification techniques have been used to generate reproducible results. Chloroplast sequences (present in high copy number), other highly repetitive genomic sequences and short microsatellite markers are analysed to increase the chance of success. Certain protocols can be used to isolate DNA markers which allow both species and variety identification.

The application of NMR together with statistical methods (i.e. chemometrics) has been used for quality assurance and to establish authenticity of foodstuffs.

There has been some success in DNA analysis of coffee beans. Briefly explain why DNA analysis is of limited applicability to roasted coffee beans.

The use of DNA based approaches can be challenging due to the fact that nucleic acids rapidly decompose during the roasting process. DNA extracted from roasted beans is fragmented and denatured making analysis difficult.

Other chemical alterations that take place during roasting provide additional barriers to DNA analysis.

Deliverable

Before conducting the laboratory investigation you will need to submit the following:

• A plan for your laboratory investigation which must be approved by your tutor/facilitator.

After conducting this investigation, you will need to submit the following:

• A report outlining your laboratory investigation including a summary of your key findings (use the template you have been given).

Unit 3: Detection and extraction of toxins in food products

Pre-session 6 preparation (for the tutor)

You need to make the following resources available to students before the session:

Email

Dear Placement Students,

The detection and extraction of chemical compounds (such as toxins and other contaminants) in food products is a hugely significant area of research for the food industry. Food imported from outside the EU can be particularly prone to toxic mould contamination resulting in the presence of unwanted toxins such as aflatoxins and mycotoxins harmful to humans and animals due to changes in climatic conditions and lack of adequate quality control with improper harvesting and storage practices. The use of insecticides and pesticides (methidathion in olive oil) is also of particular concern. Hence there is a constant need for new methods of extraction and detection of toxins and contaminants in food.

We are investigating the feasibility of adopting one new approach for extracting components from food products that we can offer to our customers. We would like you to research the following new methods and then, as a group, decide which approach is likely to be most beneficial to the company (in terms of viability, potential impact on food science/health and potential profits) and to prepare a five minute 'elevator' pitch on your chosen proposal. This pitch will be given to a panel of senior managers and will be followed by 5–10 minutes of questions.

I have given you details of some relevant journal articles that will help your research.

The areas that we are primarily interested in are:

The use of Molecularly Imprinted Polymers (MIPs) to extract and quantify hepatotoxins from samples of drinking waterWeller, M. G., "Immunoassays and Biosensors for the Detection of Cyanobacterial Toxins in Water", Sensors, 2013, 13, 15085–15112.

The use of MIPs in the extraction of kukoamine A from waste potato peels

Piletska, E. V., Burns, R., Terry, L. A., Piletsky, S. A., "Application of a molecularly imprinted polymer for the extraction of kukoamine A from potato peels", Journal of Agricultural and Food Chemistry, 2012, 60 (1), 95–99.

The use of MIPs in the extraction and analysis of pollutants in olive oil (eg methidathion)

Bakas, I., Oujji, N. B., Moczko, E., Istamboulie, G., Piletsky, S., Piletska, E., Ait-Ichou, I., Ait-Addi, E., Noguer, T., Rouillon, R., "Molecular imprinting solid phase extraction for selective detection of methidathion in olive oil", Analytica Chimica Acta, 2012, 734, 99–105. General papers:

Cheong, W.J.; Yang, S.H.; Ali, F., "Molecular imprinted polymers for separation science: A review of reviews". J. Sep. Sci. 2013, 36, 609–628.

Karim, K., Breton, B., Rouillon, R., Piletska, E. V., Guerreiro, A., Chianella, I., Piletsky, S. A., "How to find effective functional monomers for effective molecularly imprinted polymers?", Advanced Drug Delivery Reviews, 2005, 57 (12), 1795–1808.

Best wishes, Dave Ball

Senior analyst Northland Food Analysis Laboratories Ltd

Session 6 (60 – 90 minutes)

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

• The use of Molecularly Imprinted Polymers (MIPs) in the extraction and detection of specific chemical components (such as contaminants) in food products, the nature of different types of food contamination, potential applications of substances extracted from food products and the viability/profitability of adopting a new approach/technology into an existing business.

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

- Describe what a Molecularly Imprinted Polymer (MIP) is and give some details of how a MIP is synthesised
- Recognise the importance of molecular imprinting approaches in creating MIPs and be aware of approaches used to overcome the challenges of synthesising a suitable MIP for a given target
- Describe the uses of Molecularly Imprinted Polymers (MIPs) in the detection, analysis and extraction of molecules (including contaminants) from food products
- Discuss a range of food contamination issues faced by the food industry and suggest potential solutions to these problems
- Compare the use of MIPs in food contamination analysis with other approaches

TRANSFERABLE

By the end of this part of the problem students should be able to:

- Give a concise group oral presentation on a new scientific approach to a realworld problem which effectively presents the science in the context of the requirements of a profit making business
- Research a range of new scientific approaches by reviewing the peer reviewed literature
- Recognise how the requirements of a for-profit business will influence the viability of various research approaches in an industrial setting

Resources and arrangements

• This session will work best if students have access to computer facilities (eg tablet devices, laptops or PCs) with internet access which will allow them to conduct research as they tackle the problem.

Discussion questions

BACKGROUND

Briefly discuss the meaning of the term Molecularly Imprinted Polymer (MIP).

A MIP is a polymer that is synthesised using a molecular imprinting approach. This approach produces a polymer with a structure which is complementary to a given molecule.

Before the development of Molecularly Imprinted Polymers (MIPs), what methods were used to detect and/or extract contaminants from food? What are the disadvantages of these approaches?

Detection of toxins and contaminants often makes use of chromatographic (eg HPLC and TLC) and immunoassay based approaches. These approaches are somewhat time-consuming and expensive.

The use of biosensors (coupled to transducers) such as enzymes, receptors and antibodies provides excellent target specificity and selectivity but these approaches are costly, time consuming and the stability of biosensors is usually poor.

The use of biometric sensor approaches have shown much promise for the extraction and analysis of contaminants. Briefly explain what is meant by the term biometric in this context.

Biometric approaches use sensors based on artificial receptors. These sensors mimic the action of biological systems.

Briefly explain why MIPs appear to be very good potential sensors for detection of food contamination.

Molecularly imprinted polymers (MIPs) provide a low-cost, easy to synthesise sensor with high selectivity and sensitivity (dependent on the strength of the complex formed between the template molecule and the monomer). MIP based sensors can be regenerated for repeated use.

These polymers are robust, cheap and easy to make. As this is a solid phase extraction approach, the use of solvents is eliminated making this a greener solution to extraction and analysis of toxins.

Briefly describe the approaches used to create a MIP designed for a specific target.

MIPs are formed by selecting an appropriate monomer and polymerising in the presence of the desired target molecule (which acts as a template). The functional groups in the forming polymer oriented towards to the complementary functionality in the target. Once the polymer has formed, the template can be removed by extensive washing.

What approaches are used to design a monomer that will be complementary to the specified target?

Finding the optimum monomer structure for a given target may be challenging but this can be addressed by using computational approaches to model the interaction between the monomer and the target. This modelling process facilitates selection of functional polymers and allows optimisation of monomer composition.

APPLICATIONS OF MIPS

What are hepatotoxins? Why might they be present in drinking water?

Hepatotoxins are chemical toxins which cause liver damage. Both hepatotoxins and neurotoxins are produced by cyanobacteria commonly found in surface water and therefore are of relevance to water supplies (Cyanobacterial toxins: Microcystin-LR in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality. Originally published in Guidelines for drinking-water quality, 2nd ed. Addendum to Vol. 2. Health criteria and other supporting information. World Health Organization, Geneva, 1998.)

Why is the molecule methidathion sometimes detected in olive oil?

Methidathion is an organophosphorus pesticide used as a repellent for flies and insects on olive trees. In the absence of this pesticide, olive trees may be attacked and the yield of olive oil is potentially reduced. In spite of this advantage, these pesticides are highly toxic.

There are a number of existing successful methods for detection of organophosphorus pesticides in olive oils (such as GC-MS). What are the associated disadvantages with existing approaches?

Existing approaches are expensive and involve complicated sample preparation methods.

What advantages does MIP based extraction of methidathion have over existing approaches?

MIP based extraction was much more effective than traditional C18 reverse-phase solid phase extraction

Why are researchers interested in extracting kukoamine A from potato peels?

Kukoamine A is present in significant quantities in potato peels. It is known to be an important biological compound so it can be used in food supplements or pharmaceutical products (it has reported hypertensive effects and an antitrypanosomal action).

Evaluate the advantages of each of the three approaches you have researched and decide which approach would best suit the aims of the business.

All three approaches have clear benefits: the olive oil and drinking water approaches have significant health benefits. The potato peel approach has potential health benefits (from the supplements or medicines that can be made from the extracted product) plus has the added significant advantage that the extraction produces a valuable product. Students may have to think about the ethics of this decision in addition to the profitability!

Deliverable By the next session you need to:

• Research one of the specified approaches and prepare a 5 minute pitch on that approach. Be prepared to answer questions on your chosen approach. Remember to pitch your approach at a suitable level for the audience (i.e. the senior management of a for-profit scientific analysis company).

Note: you may need to define what is meant by an 'elevator pitch'. This is what we had in mind: a short (max. 5 mins), focused presentation which was delivered without the aid of PowerPoint. The presentation should highlight both scientific and business aspects of their chosen approach. We don't really expect students to present a detailed analysis of costs but we do want them to think about whether approaches can be realistically adopted by the company and what kind of return the company could potentially make. You are free to make changes to this format to suit your own expectations.

Session 7 (c.a. 15 minutes per group)

You need to use this session for the presentations. You can try to sell this as a 'Dragon's Den' type pitch – they need to thoroughly research the science and make the advantages to the company clear. They need to consider profitability, viability, etc.

Ideally each group needs to present to a panel of three people acting as senior managers. We advise you to use staff members for this activity however one staff members and two postgraduates would also be acceptable.

Pre-session preparation

Students should be prepared to discuss the following topics in this session:

- Your chosen extraction (researched since session 6) so that you deliver an elevator pitch to a team of managers.
- The scientific, ethical and business impact of your decision

Intended learning outcomes

SCIENTIFIC

By the end of this part of the problem students should be able to:

- Describe the scientific background of an extraction technique based on the use of Molecularly Imprinted Polymers (MIPs).
- Compare a range of different extraction techniques and rationalise a decision for choosing a given approach.

TRANSFERABLE

By the end of this part of the problem students should be able to:

• Verbally communicate scientific ideas to an audience of senior scientists and to respond to a range of questions on the ideas presented

Deliverable

• In this session you will deliver your 'elevator pitch'.

Outline marking criteria

Note – these marking criteria are not overly proscriptive as students can address the problems in a range of different ways.

Unit 1 – Oral presentation on analytical approaches

You can use these criteria to assess the oral presentation. You can also use these as the basis of peer-assessment of the presentations.

- Did the students summarise their technique in a concise and informative manner in the presentation?
- Did the group demonstrate a clear understanding of the scientific basis of the technique they researched? Was it clear that the students had considered the points in the discussion questions from the previous session?
- Did the students respond well to questioning? Had all of the discussion questions been addressed?
- Were the students able to discuss the strengths and weaknesses of the approach they researched?

Mark out of 10:

8 (or more) – The oral presentation was very well structured and clear. The group responded very well to all questions asked – responses were scientifically correct and delivered in a clear, confident manner. All of the points listed above were covered very well.

7 – The oral presentation was sufficiently clear to allow the listener to understand the key points made by the group. The group responded well to most questions – responses were scientifically correct and were delivered generally well. Most of the points listed above were covered well by the group's responses.

5 – The standard of the presentation was adequate. The group managed to cover some of the points listed above – The group struggled to respond to some of the questions and appeared to be 'against the ropes' at times .There may have been some scientific errors.

3 – The standard of presentation was very poor. The group struggled with most of the questions and failed to address many (or all) of the points listed above. The group struggled with most (or all) of the questions asked.

Unit 1 – Wiki

- Does the wiki provide a concise summary of the areas of background science requested in the brief?
- Does the wiki make use of appropriate labelled diagrams?
- Is there evidence that all group members have collaborated effectively on the wiki and does the wiki include appropriate hyperlinks between areas of related content?
- Has the wiki been fully referenced?

Unit 1 – Executive summary

- Does the written summary demonstrate an understanding of the scientific principles of the selected approach?
- Does the report clearly explain why this approach is suitable for this type of analysis?
- Have the strengths and weaknesses of this approach been considered?

Unit 1 – Analysis of PCR data

- Did the report include a calibration plot which was used to determine the quantity of equine DNA in the unknown samples?
- Did the report include an explanation of how the data was generated (i.e. by recording the cycle number at which the fluorescence values exceeded a threshold value?).
- Did the report consider the limitations of this approach in terms of generating a quantitative conclusion?
- Did the report identify the fact that the only samples that tested positive were canned meat reserves that may have been prepared before current legal measures to prevent phenylbutazone from entering the human food chain had come in to effect?
- Did the report include a reasonable conclusion (some limited evidence that equine DNA has been found in consumer products in Northland. Recommendation of immediate withdrawal of products and further, widespread testing) and sensible recommendations for future work (eg further, more extensive testing of consumer products for equine DNA)?

Unit 2 – Experimental plan (vodka)

- Did the plan clearly describe how the experiment was to be conducted? Could someone else follow the plan and successfully run the experiment?
- Did the plan include a list of required equipment and reagents?
- Was an indication of the anticipated results included? Did students show how to quantify the result (if possible)?

Unit 2 – Experimental plan (coffee)

- Did the plan clearly describe how the experiment was to be conducted? Could someone else follow the plan and successfully run the experiment?
- Did the plan include a list of required equipment and reagents?
- Did the students give some idea of what type of data the experiment would produce?

Unit 2 – Experimental report (coffee)

- Does the report include a discussion of the viability of the investigated method(s)? Did the group(s) discuss reproducibility of results?
- Did the investigation include a variety of different approaches?
- Have the results been fully described? Have charts, plots and spectra been quoted where appropriate?
- Does the report include conclusions and recommendations based on what was learnt?

Unit 3 – Oral presentation on MIP based extraction

You can use these criteria to assess the oral presentation.

- Did the students summarise the approach in a concise and informative manner in the presentation?
- Did the group demonstrate a clear understanding of the scientific basis of the technique they researched? Was it clear that the students had considered the points in the discussion questions from the previous session?
- Were the students able to rationalise the basis of their decision?
- Did the students consider the profitability and viability of their approach?
- Did the students respond well to questioning? Had all of the discussion questions been addressed?

Mark out of 10:

8 (or more) – The oral presentation was very well structured and clear. The group responded very well to all questions asked – responses were scientifically correct and delivered in a clear, confident manner. All of the points listed above were covered very well.

7 – The oral presentation was sufficiently clear to allow the listener to understand the key points made by the group. The group responded well to most questions – responses were scientifically correct and were delivered generally well. Most of the points listed above were covered well by the group's responses.

5 – The standard of the presentation was adequate. The group managed to cover some of the points listed above – The group struggled to respond to some of the questions and appeared to be 'against the ropes' at times .There may have been some scientific errors.

3 – The standard of presentation was very poor. The group struggled with most of the questions and failed to address many (or all) of the points listed above. The group struggled with most (or all) of the questions asked.

Written content

- A (1st) Excellent. The answer contains all the things listed in the criteria and one or two extra related things. All numerical values have been correctly calculated and are presented with units and discussion.
- B (2.1) Very Good. The answer has almost all the things listed in the criteria (only minor things missing) any calculated values are close to the recommended values.
- C (2.2) Good. Most of the things listed in the criteria appear in the answer, some missing content and/or some error in written content and/or calculations.
- D (3rd) Modest. The answer has significant content missing. There are a number of errors throughout the answer.
- E (Fail) Poor. The answer contains only a few of the important points from the list. There are significant errors and missing sections of content.

Presentation of written solutions

- A (1st) Excellent. The solution is very well structured and produced. It is easy to find the different sections and there is similar presentation on different pages. Each section is virtually free from errors in grammar, spelling and punctuation and makes good use of referenced and labelled diagrams.
- B (2.1) Very good. The solution is well structured and produced. It is clearly written apart from relatively minor aspects which would not seriously affect the understanding of the reader. The solution makes good use of referenced and labelled diagrams to clarify key points in the answer.
- C (2.2) Good. Though reasonably well structured and produced, the solution contains significant errors in grammar and spelling. Diagrams were provided, these were not always referenced and/or there was little attempt made to relate these to the answer.
- D (3rd) Modest. The solution was disorganised and disjointed and so badly produced that it would inevitably misguide the reader. There were lots of errors spelling, grammar, lots of different fonts and little or no evidence of teamwork. There was little or no effort to provide diagrams or examples to illustrate points in the answer.

Investigation of methods to detect adulteration of coffee

Northland Food Analysis Laboratories Ltd Northchester Northland

Date (Month, Year)

Referencing and acknowledgements

Executive summary Maximum one page, Arial, 12pt text

Report

Maximum 4 pages, Arial 12 point text, divided into the following sections.

Background

Methods

This section should include a brief discussion justifying why each method was used and how data was generated from these approaches.

Analysis of results

This section may include charts and tables. You may also discuss limitations of results here.

Conclusions

• Bullet point list.

Recommendations for actions based on results

Recommendations for future work

Analysis of meat products for presence of equine DNA

Northland Food Analysis Laboratories Ltd Northchester Northland

Date (Month, Year)

Referencing and acknowledgements

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• Bullet point list.

Recommendations for actions based on results

Recommendations for future work