BRITISH PHARMACOPOEIA COMMISSION

Working Party (DNA): Identification Techniques

SUMMARY MINUTES

A meeting of this Working Party was held at 151 Buckingham Palace Road, London, SW1W 9SZ on Wednesday 21st January 2015.

Present: Dr K Helliwell (Chairman), Dr I Feavers, Dr J A Hawkins, Dr E Mee, Prof. A. Slater and Prof. E M Williamson.

In attendance
NIBSC: Dr Caroline Howard, Mrs C Lockie-Williams
BP Secretariat: Dr P Holland and Mr M Whaley.
Ms H Corns (BP Secretariat) gave a short presentation on Data Integrity.

MINUTES

9.0 The minutes of the meeting held on the 25th November 2011 were confirmed subject to the following amendment.

Minute 6.0: Ocimum Tenuiflorum
Last paragraph: replace the penultimate sentence by the following.

Professor Slater added that he had some seeds of *O.tenuiflorum* which were not held under an MOU and also had some plants of *O. gratissimum* which could be accessed for this project.

MATTERS ARISING FROM THE MINUTES

10.0 There were no matters arising from the minutes.

11.0 **Data Integrity Presentation**
Ms Corns gave a short presentation on 'Data Integrity'. It was noted that the Secretariat was in the process of restructuring the BP website and the Working Party (DNA): Identification Techniques will have a dedicated forum. Members were asked to register for access to the forum.

12.0 **Terms of Reference** WP(DNA) (15)01
A copy of the Terms of Reference (ToR) for the Working Party was presented. Members were invited to reflect briefly on whether the expertise within the Working Party would enable the Secretariat to deliver the expectations outlined in the Terms of Reference document in the near future. There was consensus that the expertise within the Working Party was aligned with the objectives stated in the ToR.

13.0 **Membership Process** WP(DNA) (15)02
A paper on the process for appointing members to the BP Commission Expert Advisory Groups, Panels of experts and Working parties was presented. The list of proposed members as well as their areas of expertise, with Dr Helliwell as Chairman was noted. The initial appointments of members were for 2 years.

This will be reviewed in line with the next phase of the BP-NIBSC herbal project.

The diligent and timely contributions from members, their willingness to work towards understanding the objectives of the herbal project and in providing advice were acknowledged. This had enabled the MHRA to achieve the objectives pertaining to establishing a DNA-based identification test method for herbal drugs within the Terms of Reference. The Chairman in particular had been instrumental in helping the MHRA to achieve the objectives within a very tight schedule. All of the above had been achieved whilst ensuring new pharmacopoeial initiatives will not overburden the herbal industry.

Members attention was drawn to the confidential nature of matters discussed by the Working party, in particular the commercial sensitivity of information relating to the innovative ideas and products under consideration by the MHRA.

Members were asked to register to the BP website where they could access directly documents for comment and information, and papers for meetings. This is part of the risk management strategy to mitigate the possibility of losing confidential data.

14 **Herbal Project** WP(DNA) (15)03

Objectives The two broad objectives of the molecular biology arm of the herbal project were noted, namely (a) authentication of commercial herbal drug samples using DNA barcoding and other relevant techniques and (b) DNA-based identification methods for inclusion in BP published monographs, where this adds value. Members were mindful of not overburdening the UK herbal industry.

The first draft guidelines on 'DNA barcoding as an identification method in a BP Monograph' was discussed and the following comments were made. A revised document will be presented to EAG HCM at its meeting scheduled in June 2015 for comment. At the WP (DNA) meeting in June 2014, it was agreed that in the absence of voucher samples the BOLD database was a reliable substitute but advised caution in respect to other available databases. The use of sequences from more reliable databases, such as BOLD was endorsed with the caveat that caution should always be exercised.

DNA-based method as a replacement of the microscopical descriptions routinely included in pharmacopoeial monographs for identification was discussed. Prof. Williamson commented that no test stood alone in a pharmacopoeial monograph. The monograph could be structured to give the analyst a choice on whether to do microscopy or DNA-barcoding identification test. Dr Helliwell added that microscopical examination would be a better tool for confirming the plant part. DNA barcoding was more sensitive to distinguishing between the monograph subject and adulterants, substitutes and contaminants. It provides unequivocal information on the botanical identification of the material. There was some discussion about designing DNA barcoding methods to detect contaminants at a level of less than the accepted pharmacopoeial limit of not more than 2% of foreign matter. The point that the safe level of toxicity would be different for each species was accepted. And also that it was important to recognise all adulterants and substitutes especially those that are toxic, when making a decision on what constitutes as a safe limit. The consensus was that for some toxic substances it would be worthwhile

researching the suitability of DNA barcoding as a test method for instance as means of detecting Aristolochia species. It was suggested that herbs known to contain aristolochic acid could be routinely screened at the BP-NIBSC herbal laboratory.

In respect to the possibility of using DNA barcoding to distinguish between the monograph subject and powdered material of animal origin, the consensus was that this was not necessary as UK legislative requirements prohibited addition of material of animal origin to herbal medicines.

It was accepted that barcode data generated during the authentication process of herbal drugs should be presented in a BP supplementary chapter. Another possibility would be to make the barcoding information on the BP website more interactive to engage users.

Members discussed whether verification of a proposed BP method and/or data produced at a second laboratory will be a necessary requirement for all cases. It was accepted that there was value for a second laboratory verification of the first methods developed at BP-NIBSC herbal laboratory. However over time and where the method used was a simple Sanger sequencing based barcoding technique, the usefulness of a second laboratory verification was limited. It was confirmed that for a simple Sanger sequencing-based barcoding technique, a second laboratory verification of sequence generated at the BP-NIBSC herbal laboratory was not needed. Verification would be required for a qPCR method. Consensus was that for quantitative methods a second laboratory verification of BP data would be worthwhile because there is more possibility of an effect from the analyst due to differences in technique or the reagents used. It was noted also that the desired outputs of purity assignments are also less stable, looking for differences between 92% and 95% purity rather than the probability of a base identification being correct or incorrect.

15 **Monographs in Progress** WP(DNA) (15)04

A list of monographs of herbal drugs currently being progressed by EAG HCM was presented. WP (DNA) members were invited to forward information they may have on the listed herbs to the Secretariat for consideration.

Squill Dr Helliwell raised concerns about compliance of items of commerce (white squill) with the published BP monographs for Squill; defined in the monograph as consisting of the bulb of *Drimia maritima* (L.) Stearn. It was grown in the Mediterranean and evidence showed that species other than *Drimia maritima* (L.) Stearn were sold as white squill. He undertook to supply the BP-NIBSC herbal Laboratory with commercially available samples for barcoding analysis. The monographs for Squill and that for Squill Liquid Extract will be reviewed when the barcoding information for Squill was available. Dr Hawkins offered to provide the BP-NIBSC herbal Laboratory with herbarium samples of *Drimia maritima* (L.) Stearn and other closely related species.

16 **Implementation of DNA-Based Identification Methods for Herbal Drugs** WP(DNA) (15)05

A revised draft document detailing general proposals for a phased introduction of DNA based testing methods in the BP 2016 was received. The document had been prepared in consultation with the Chairman and reflected previous comments by members agreed by the Working Party. Members were invited to

send the Secretariat any further comments they may have by the agreed deadline. The Chairman specifically asked members to verify the definitions of the terms in the Glossary table. Consensus was that a glossary would be beneficial to users. The following comments were made at the meeting.

Barcoding Publication Platform Members discussed the advantages and disadvantages of different publication platforms for the barcode sequences. The consensus was that presentation on a webpage would lend itself to ease of frequent updates whereas this would not be the case if included in a published book. It was noted that some BP users had a preference for the hardcopy. Consensus was that both platforms could be used where the information was updated annually within the hardcopy if warranted and frequent webpage updates if needed. For clarity, the key areas within the barcoding sequence would be formatted differently from the other DNA bases. Compliance requirement was for a 100% match of the key bases. For the non-key bases within the sequence, it was discussed what the threshold would be for samples compared with the reference sequence. It was explained that the intention was to keep PCRs as universal as possible, so protocols would be specific to each region, as for instance *trnH-psbA*, rather than a species. As was suggested by Dr Mee, it was agreed to publish a reference sequence which could be downloaded by users of the Pharmacopoeia.

Aims for BP 2017: DNA Test Methods The options presented in the revised draft guidelines were discussed in the context of further developmental work, the results of which could be published in the BP 2017. A preference was to investigate further the application of qPCR coupled with high resolution melting for the analysis of herbal drugs, as this would lend itself particularly to the sequence differences seen in *Ocimum tenuiflorum*. Service provision to industry was discussed in the context of financial sustainability of the herbal project. Dr Hawkins's suggestion of the MHRA providing a service to the industry together with corresponding standards was discussed. This was instead of a published test method within the Pharmacopoeia. It was accepted that provision of a service could be more appropriate and more controllable. Legal advice would be sought on the appropriateness of providing a service if it was likely that the results would be used as part of the dossier for a licence/registration application under the auspices of the MHRA. Members also considered the use of dedicated kits including positive and negative controls. The BP-NIBSC project team will examine the practicalities of different implementation strategies. The outcomes will be presented for further consideration at a future meeting.

Second Laboratory Testing Verification De Montfort University had been asked to measure the robustness and transferability of the test method that had been designed and used at the BP-NIBSC herbal Laboratory to analyse commercial samples of *O. tenuiflorum*. Professor Slater presented the results. He said the requirements outlined in the BP-NIBSC Laboratory protocol were appropriate and that the laboratory requirements were met by the facilities at De Montfort.

It was noted that the results were significantly similar between the two laboratories. The method and depth of sampling was found to be of importance with discussion raised regarding the duplicate/triplicate DNA extractions per sample, and the requirement for three reads of the subsequent PCR products. Three reads for every PCR product was felt to be demanding and expensive, particularly where multiple DNA extractions had been performed. The

suggestion for PCRs to be performed in duplicate from one representative DNA extraction, coupled with two subsequent sequencing reads was thought to be more than adequate for the Appendix method. Professor Slater suggested that it would be useful to assess the qPCR model in place for detecting and quantifying genetically modified organisms (GMO) in food, as a model for the use of qPCR in herbal drugs. The question of sample milling was raised in the context of whether adulteration of substitution would be missed in a large amount or over highlighted in a small volume sample. It was agreed to follow the instructions within the BP Appendix XI T. Herbal Drugs: Sampling and Sample Preparation for future barcoding work.

17 **Barcode Reference Material** WP(DNA) (15)06

The publication of test and assay methods within the BP was usually accompanied by provision of materials enabling the user to ensure the methods were performing correctly. Members considered the details of the design of the barcode reference material which could be used to prove system suitability when performing DNA identification tests.

Nomenclature The proposed nomenclature for the reference material 'BP Barcode Reference substance/BP Barcode Reference Material was discussed briefly. A preferred option was to adopt BP Nucleic Acid Reference Material (BPNARM).

Design and Production of the Reference Material It was noted that 'fitness-for-purpose' studies will be conducted at laboratories at NIBSC prior to accelerated degradation studies. The expectations were for no degradation of the material in the first 6 months and for it to be stable in Gentegra tubes up to 4 years. 30 individual tubes will be used for the fitness-for-purpose studies. Legal advice will be sought about possible infringement of the biodiversity act in regards to profiting from the sale of genetic material. Consensus at the meeting was that the design of the reference material was a synthetic construct and infringement was unlikely.

18 ***Ocimum tenuiflorum*** WP(DNA) (15)07
Ocimum gratissimum

The draft monograph for Holy Basil Leaf to be included in material for publication in the BP 2016 defined it as *Ocimum tenuiflorum* L. It was understood that under Ayurveda three different forms of Tulsi, Holy Basil, were recognised; two of which were *O. tenuiflorum* and the other *O. gratissimum*. The composition of the published 2016 monograph will be reviewed in time for any necessary revision to the monograph by means of the BP 2017, subject to the availability of samples of *O. gratissimum* and the similarity or otherwise of the chemistry of the two species. Prof. Williamson commented that there were distinct morphological differences between the two species. She undertook to examine samples of *O. gratissimum* with the view to confirming the available information on the diagnostic differences between *O.tenuiflorum* and *O.gratissimum*. Prof. Slater commented that there were uncertainties about nomenclature of *O. gratissimum* and offered support on how the two species can be separated. Dr Howard commented that there were multiple sequences for *O. gratissimum* in the BOLD database, and that these align differently. There were differences in barcoding between *Ocimum* species. Dr Hawkins raised the possibility of hybrids and interbreeding between species.

19 **Holy Basil Leaf** WP(DNA) (15)08

Members considered the added value of specifying a DNA barcoding identification method in the monograph for Holy Basil Leaf to be included in material for publication in the BP 2016. Given the intention was to introduce a qPCR identification method for Holy Basil Leaf, ideally, by means of the BP 2017, it was agreed that no added value would be gained by introducing a barcoding 'yes or no' identification method in the BP 2016 monograph. Furthermore the project feasibility test had been achieved regardless of positioning of the barcode of *Ocimum tenuiflorum* within the BP publication. It was agreed to include a general appendix method for DNA barcoding of herbal drugs with the DNA barcoding method for *Ocimum tenuiflorum* as an adjunct to the appendix. Prof. Slater queried whether Holy basil Leaf would be the best candidate for qPCR in terms of providing a useful method for industry. Further consideration would be given to selecting a herbal drug suited to demonstrate proof of concept of qPCR application.

20 **Phellodendron Amurense Bark** WP(DNA) (15)09
Phellodendron Chinense Bark

A full report was presented detailing the results of the study on *Phellodendron chinense* and *Phellodendron amurense*. The results mirrored some of the morphological information received from the Royal Botanic Gardens, Kew, but also showed that some samples were mixed and others contained contaminating material.

The outer tree bark resulted in a low yield of DNA but no sample clean-up was necessary. Identification of the different species was challenging but ultimately successful when using the *trnH-psbA* region. Attention was drawn to the 'conclusions and outputs' in the report. The contaminants identified for two separate situations appeared to be from the genera *Populus* and *Quercus* which were unexpected. The possibility of including the barcoding sequences of amurense and chinense in the BP 2016 monographs to differentiate between the two species was considered. Members accepted the Secretariat's preferred option to publish the results by means of the BP 2017 as part of a new supplementary chapter. The BP 2016 monographs contained a chemical-based differentiation test method for the 2 species.

21 **Work in Progress at the BP-NIBSC** WP (DNA) (15)10
Herbal Laboratory

Mrs Lockie-Williams updated members on the current laboratory activities. The BP-NIBSC herbal Laboratory were currently performing DNA-based analysis of *Ophiopogon japonicus*, *Anethum Graveolens Sowa*, *Phyllanthus amarus* and *Passiflora incarnata* aqueous extract.

Ophiopogon japonicus The items of commerce were in the form of tubers and the work was at the PCR stage. It was noted there was a 50% success rate for the amplification of the DNA extracted from the samples. The work was on-going. It would be helpful to ascertain from the suppliers information on the routine processing methods applied to the tubers.

***Anethum Graveolens* Sowa** The work was on-going. A full report will be presented to the Working Party (DNA): Identification Techniques at the earliest opportunity.

Phyllanthus amarus The samples received were of varied plant parts and contained some foreign matter. A full report will be presented to the Working Party (DNA): Identification Techniques at the earliest opportunity.

***Passiflora incarnata* aqueous extract**
A single *Passiflora incarnata* sample had been provided by the Chairman. Progress of the on-going work was noted. It was commented that herbarium voucher samples for *Passiflora* were available at the University of Reading Herbarium. There was a National Collection of *Passiflora* at the herbarium. It was advised that a newly marketed 'Qiagen kit- DNeasy mericon Food Kit' for processed food stuffs was useful for samples with degraded DNA and this may be useful for this work.

22 **Any Other Business** WP (DNA) (15)11
It was noted that a paper on the feasibility of the herbal project will be presented to BP senior management for consideration.

23 **Meeting Dates in 2016**
Thursday, 28th of January
Wednesday, 20th of July

Acronym/Synonym	Name
APhI	Ayurvedic Pharmacopoeia of India
ATA	Ayurvedic Trade Association
BHomP	British Homoeopathic Pharmacopoeia
BP	British Pharmacopoeia
BP (Vet)	British Pharmacopoeia (Veterinary)
BP Commission	British Pharmacopoeia Commission
BPCx	British Pharmaceutical Codex
BPCRS	British Pharmacopoeia Chemical Reference Substance
BS	British Standard
BHMA	British Herbal Medicine Association
BOLD	Barcode of Life Database
CMPACC	Chinese Medicinal Plants Authentication and Conservation Centre (Kew)
CEP	Certification Procedure for the European Directorate for the Quality of Medicines
CHM	Commission on Human Medicines
CP	Pharmacopoeia of the People's Republic of China
CRS	Chemical Reference Substance
DNA	Deoxyribonucleic Acid
EAG	Expert Advisory Group
EPC	European Pharmacopoeia Commission
EPCRS	European Pharmacopoeia Chemical Reference Substance
EU	European Union
FDA	Food and Drug Administration
FIP	International Pharmaceutical Federation
FoI	Freedom of Information
GC	Gas chromatography
HAB	German Homoeopathic Pharmacopoeia
HCM	Herbal and Complementary Medicines
HKCMMS	Hong Kong Chinese Materia Medica Standards
HMPC	Herbal Medicinal Products Committee
ICH	International Conference on Harmonisation

IR	Infrared
ISO	International Organisation for Standardisation
JP	Japanese Pharmacopoeia
LC	Liquid chromatography
LD	Licensing Division
LGC	Laboratory of the Government Chemist, Teddington
LR	BP Laboratory Report
MAIL	Medicines Act Information Leaflet
MHRA	Medicines and Healthcare products Regulatory Agency
MPNS	Medicinal Plant Names Services - Royal Botanic Gardens, Kew
NIBSC	National Institute for Biological Standards and Control
NPA	National Pharmacopoeial Authority
OMCL	Official Medicines Control Laboratory
Ph Eur	European Pharmacopoeia
PMU	Pharmacy Medicines Unit – to be confirmed
QSIMP	Quality Standards of Indian Medicinal Plants
SOP	Standard Operating Procedure
SPC	Special Product Characteristics
TGA	Therapeutic Goods Administration, Australia
THMPD	Traditional Herbal Medicinal Products Directive
TLC	Thin layer chromatography
UK	United Kingdom
UKD	United Kingdom Delegation [to the European Pharmacopoeia]
USP	United States Pharmacopeia
UV	Ultraviolet
WHO	World Health Organization