BRITISH PHARMACOPOEIA COMMISSION

Working Party (DNA): Identification Techniques

SUMMARY MINUTES

A meeting of this Working Party was held at 151 Buckingham Palace Road, London, on 28th of January 2016.

Present: Dr K Helliwell (Chairman), Dr I Feavers, Dr E Mee, Prof. A. Slater and Prof. E M Williamson.
In attendance
NIBSC: Dr C Howard, Mrs C Lockie-Williams, Mr L Gibson.
BP Secretariat: Dr P Holland, Dr R Pask-Hughes and Mr M Whaley.

An apology for absence was received from Dr J A Hawkins.

41 **INTRODUCTORY REMARKS**

Welcome The Chairman welcomed members to the meeting, in particular Mr Gibson who was attending his first meeting. Mr Gibson is an Executive Officer based at the BP-NIBSC Herbal Laboratory.

Declaration of Interests No conflicts of interest were declared.

Confidential The papers, discussion and the minutes of the meeting were noted to be confidential.

I MINUTES

42 The minutes of the meeting held on 10th of July 2015 were confirmed subject to a minor editorial amendment.

II MATTERS ARISING FROM THE MINUTES

43 A list of matters arising from the minutes of the meeting of the WP (DNA) held in July 2015 was circulated together with the papers for this meeting. A copy is appended.

In addition to the items listed, members were updated on laboratory projects including *Galium aparine*, *Drimia maritima*, *Myristica fragrans*, and the purchase and installation of a qPCR machine. Members welcomed the update and anticipated the publication of full reports in due course.

44 **Membership** WP (DNA) (16) 01

Members had been appointed for 2 years with the term of office ending in December 2016. In view of the decision by MHRA management to extend the allocated time of the herbal project by a further 3 years (ca. March 2020), it was proposed to extend the term of office of members of the WP (DNA) to end in December 2018 bringing them into line with members of other BP Commission EAGs, Panels and WPs. The proposed extension to the term of office of members will be drawn to the attention of the BP Commission for approval.

Members were informed that 'Working Party' status was assigned to a BP

Commission group of experts with responsibility to deliver a defined project over a distinct period of time. With the extension of the herbal project from 3 to 6 years, Commission would asked to change the status of WP (DNA) to a Panel of Experts [Panel DNA: Identification Techniques].

45 **Work Programme** WP (DNA) (16) 02

The work programme of EAG HCM was presented. The importance of demonstrating increased outputs year-on-year by maximising the use of the additional resources made available by MHRA management for the herbal project was noted. The major factors impacting on the rate of development of new and revised BP monographs for herbal drugs were noted.

Herbal drug samples The difficulty in gaining access to quality herbal drugs had been discussed at the meeting of EAG HCM in November 2015. The Secretariat had been keen to adopt a pragmatic approach in obtaining samples for monograph development work. The Secretariat was working with solicitors to put in place 'Material Transfer Agreement' which should set out any limitations of use of herbal materials provided by partners and collaborators. It was commented that for the quality assurance of samples used for monograph development, the Secretariat should aim to work with members of HCM and the WP (DNA) to source samples that were likely to be GMP compliant.

46 **BP-NIBSC Laboratory Processes Update** WP (DNA) (16) 03

Members were provided with details of a change in the production of Sanger sequencing data for the project. Previously this had been accomplished within the laboratory using an existing Sanger sequencing machine. Machine errors had hindered progress and the equipment was no longer reliable, for this reason the group were now outsourcing this work to an external provider. It was explained that this was a sustainable method of data production as the cost differed little from the in-house method and service levels from the selected company were high. WP members endorsed the change in approach to production method of Sanger sequencing data.

47 **Stability and Shelf-life Testing of the *trnH-psbA* BPNARM** WP (DNA) (16) 04

A plan to provide data as to the shelf-life of the *trnH-psbA* BPNARM was presented. Details were given of the plan to test samples stored at ambient and elevated temperatures, providing data on real-time and accelerated degradation respectively. It was noted that, like other available BPCRSs a 3-month shelf-life had been assigned to the BPNARM. It was agreed that the current shelf-life of three months was short and data to support an increase in the shelf-life could be of use. It was suggested that data should be presented after two years of the planned five year study. A member questioned whether there would be any value to ascertaining further detail on degradation, as measured by qPCR methods. Consensus was that as the material was to be used in an end point assay, then this should be the test for degradation also. The proposed plan was endorsed. The first set of results will be reported at the earliest opportunity.

48 **Generating Reference Sequences for *Ph. Eur*. Herbal Drugs** WP (DNA) (16) 05

The agreed policy was to use DNA barcoding routinely to authenticate herbal drugs used for the development of test and assay methods for

inclusion in BP monographs for herbal drugs. In the case when there is agreement by EAG HCM and the WP (DNA) that a DNA barcoding based method adds value to determining the quality of the herbal drug it will be specified as an identification method in the monograph of the herbal drug. Barcoding data generated at the BP-NIBSC herbal Laboratory not included as an identification method in the monograph will be included in BP publications as part of a dedicated Supplementary Chapter on barcoding of herbal drugs.

Members considered the benefits of generating barcode data for herbal drugs which were the subject of Ph. Eur. monographs for inclusion in the proposed BP Supplementary Chapter on barcoding of herbal drugs. Including DNA barcodes for herbal drugs which were the subject of Ph. Eur. monographs that have relevance to the UK and countries where the BP was an inherent part of medicines legislation should have a positive impact on the quality of herbal medicines. The validity of herbal drug samples used for the barcoding would be shown by compliance with the corresponding published Ph. Eur. monograph. These samples would then be subject to barcode analysis.

49 **Supplementary Chapter** WP (DNA) (16) 06

A draft supplementary chapter titled 'DNA Barcoding as a tool for Botanical Identification of Herbal Drugs' was presented. Attention was drawn to the legal distinction between the requirement for users to demonstrate compliance with information within published monographs and appendices and the fact that BP published supplementary chapters are for guidance and for information. The consensus was that the draft supplementary chapter should be amended to include more information explaining the basis of DNA barcoding as an identification tool for herbal drugs. The Chairman undertook to amend the draft text in consultation with the Secretariat. The finalised draft text will be presented to the BP Commission for approval at its meeting scheduled in March 2016.

50 ***Anethum graveolens* Sowa** WP (DNA) (16) 07

A full report on the analysis of *Anethum graveolens* Sowa samples was presented at the meeting the WP (DNA) in July 2015. Members accepted the proposal by the BP-NIBSC Herbal laboratory to continue work to further substantiate an initial finding of a sub-species specific sequence in the ITS region. Data that had been produced and analysed which concentrated on completing the data set for commercial samples, and sourcing and analysing non-Sowa *Anethum graveolens* samples was presented. The data were produced by amplification and sequencing of the smaller ITS2 region, which was easier to amplify and covered the bases of interest well. Members considered the data of the final analysis and agreed that it showed good consensus with the full report provided. Members endorsed the findings.

51 ***Ophiopogon japonicus*** WP (DNA) (16) 08

At the meeting of the WP (DNA) in July 2015, members were provided with a full report of the analysis of *Ophiopogon japonicus* samples. The conclusion of that report was that the ITS region was capable of distinguishing *O. japonicus* tubers from those of close relatives and likely adulterant species, this was of particular interest due to the morphological similarity of *O. japonicus* and *Liriope spicata* tubers. However, 50% of the samples tested yielded no useful DNA and this finding correlated with a

difference in appearance of the tubers. The BP-NIBSC Laboratory had reported that investigation of the possible cause of this had been conducted with comment provided from an expert in Traditional Chinese Medicine and a reputable supplier of TCMs in the UK. Both parties had confirmed that non-reputable suppliers used various processing methods such as 'sulphating' and 'spraying with powdered Cinnabaris', Dr Howard explained that both of these procedures would damage DNA on the surface of the tubers. Members agreed that this was the likely cause of the findings in the report presented at the meeting July 2015. It was agreed that the observations reported by the BP-NIBSC Laboratory could be published, at such time as there was a Ph Eur monograph for this subject.

52 ***Vitex negundo* Leaf** WP (DNA) (16) 09

16 samples had been analysed by the BP-NIBSC Laboratory, 13 of *Vitex negundo* and 3 of *Vitex agnus-castus*, samples were dried leaves and stems with some fruit present. The BP-NIBSC herbal laboratory had conducted a literature review of published sequence data for *V. negundo* and *V. agnus-castus*, both medicinal species and potential adulterants of one another, and *Vitex trifolia*, a close relative. The available published data were used to highlight barcode regions that showed the potential to be species-specific to *V. negundo*, and those that did not and could therefore be excluded from further analysis. Data available for *rbcL* and *matK* regions showed that these regions should be excluded from analysis, and as only two published sequences were available for *trnL-F* this region was held in reserve but not investigated at this time. The ITS and *trnH-psbA* regions both showed promise to be species specific, so amplification and sequencing of these regions was attempted from all samples. DNA extraction, amplification and sequencing were straight forward, requiring no alterations to the established methods used at the BP-NIBSC Laboratory. Due to the potentially mixed nature of the samples, 3 DNA extractions were performed from each commercial sample and analysed individually.

Although sequencing was straight forward, samples from this analysis had been included in the machine errors encountered so a full data set was not available for the ITS region. Further data is required to investigate this finding.

Sequences from the *trnH-psbA* region were produced for nineteen DNA samples, from 15 of the plant samples provided. A three base polymorphism and a two base insertion/deletion (indel) separate samples categorised as 'Good match according to an independent dataset' from 'No match samples' and those from *V. agnus-castus* samples. 'Reasonable Match' samples were divided between the two groupings. This finding enabled the BP-NIBSC Herbal Laboratory to create a reference sequence, highlighting the species-specific bases as key for identification.
However, on comparison with published sequences it transpired that the reference sequence, showing 'Good Match' samples did not align with sequences published as *V. negundo*. The reliability of published sequence data is highly questionable, and many examples of mislabelled sequences have been highlighted. Due to this the results was presented to the Working Party members for their comment, and recommended that work continue to sequence vouchered samples of *V. negundo* and also to analyse the ITS region fully. Members discussed the reliability of published sequence data. A member commended the work of the BP-NIBSC Herbal Laboratory and highlighted the complex morphology and phylogeny of the *Vitex* genus. It

was suggested that further investigation of the literature in this field could yield useful information. Members endorsed the planned course for continuation of this work at the BP-NIBSC Herbal Laboratory.

53 **Planned Revisions to Appendix XI V - (DNA) Based Identification Techniques for Herbal Drugs** WP (DNA) (16)10

Members agreed to the proposal to use 20 mg of the herbal drug being analysed for the DNA Extraction (Step 5 in the published appendix method). A quantity of 200 mg is currently specified in the published appendix method XI V.

54 **Any Other Business**

Trial formatting of Papers of the meeting As had been requested feedback on the helpfulness of the new format of papers for this meeting was received.

Matters Arising (cont.,) Tabled Paper Working Party (DNA): Identification Techniques BRITISH PHARMACOPOEIA COMMISSION	
Minute 27 Terms of Reference (TOR)	The revised TOR will be drawn to the attention of the BP Commission at its meeting in March 2016.
Minute 28 Guidelines – The use of DNA barcoding as an identification method in BP monographs	The revised guidelines will be drawn to the attention of the BP Commission at its meeting in March 2016
Minute 30 Deoxyribonucleic Acid (DNA)-Based Identification Techniques for Herbal Drug Preparations and Herbal Medicinal Products	The BP-NIBSC Laboratory to design an investigation into the applicability of DNA testing to the variety of herbal preparations on the market at the earliest opportunity. Members to be invited to comment on the design of the investigative work to be done by correspondence. This will be progressed at the earliest opportunity.
Minute 31 **Operational and Strategic Plans** The Secretariat and BP-NIBSC Herbal Laboratory, in consultation with the Working Party (DNA) to decide a target herbal drug for qPCR. A document listing the strengths and weaknesses of each target for comment by correspondence should facilitate the discussion. The Secretariat to obtain HPTLC analysis data from a reputable company specialising in HPTLC on *Echinacea spp*.	There are on-going discussions to establish an 'Agreement on working with DMU'. Further discussion on sharing information on Black Cohosh will be progressed when the legal agreement is place. This will be progressed at the earliest opportunity.
Minute 34 *O. tenuiflorum* *O. gratissimum*	The analytical work would be continued subject to acquisition of the requisite number of batches of *O.gratissimum*. No batches have been sourced to date.
Minute 38 Sanger vs. NGS – *Phyllanthus amarus*	A first attempt at data analysis was completed by the Bioinformatics lead at NIBSC but requires much further work before it is applicable. Work will be progressed at the earliest opportunity.

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
ICMM (China)	Institute of Chinese Materia Medica

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
qPCR	Quantitative Polymerase Chain Reaction
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
TCM	Traditional Chinese Medicine
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