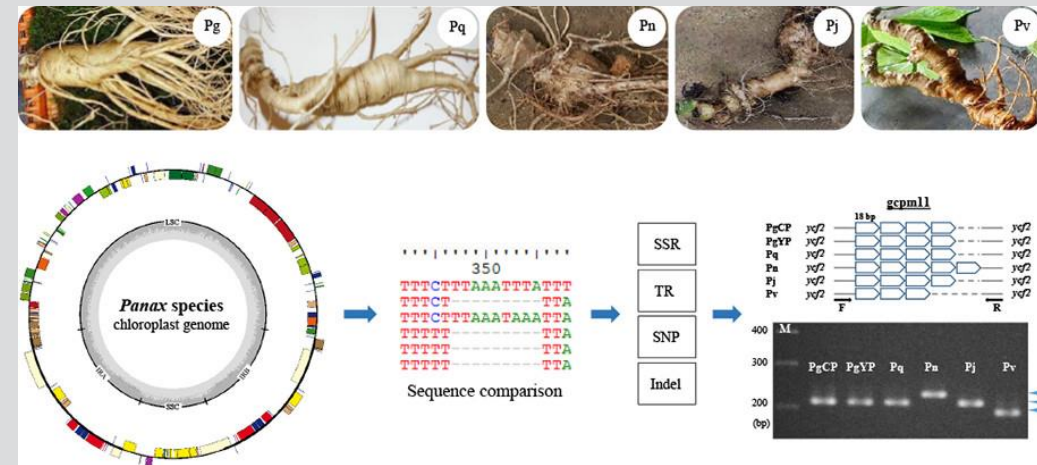


USP Dietary Supplements Stakeholder Forum
Tuesday, May 15, 2018

DNA Methods for Botanical Identification

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Jeremy Stewart, Ph.D., Gaia Herbs





Discussions regarding DNA methods for botanical identification:

- ▶ Industry asked USP to take a lead role in exploring development of a repository of authenticated plant material that could serve validation purposes in DNA procedures.
- ▶ Industry is looking for partnerships to ensure that if a DNA method is developed as a standard, it identifies material representative of articles in commerce.
- ▶ USP could take the lead in consolidating information from the various DNA libraries into a single repository targeted for dietary supplements as a reliable resource for researchers and ingredient purchasers.
- ▶ DNA-based methods would complement the current USP chemical and botanical methods for the identification of these articles. USP general chapter best-practice guidelines, monograph methods and reference standards would bring transparency to botanical quality controls.

What We Heard – 2017 USP Dietary Supplement Stakeholder Forum



Some laboratories refuse to test extracts, given loss of DNA integrity.

- DNA testing of extracts can sometimes be done on a case-by-case basis; however, DNA could be degraded and therefore less amenable to amplification in extracts.

DNA testing is an evolving science and not presently recommended as a stand alone ID test method.

DNA-based methods are complex and require a manageable number of samples.

What We Heard – 2017 USP Dietary Supplement Stakeholder Forum

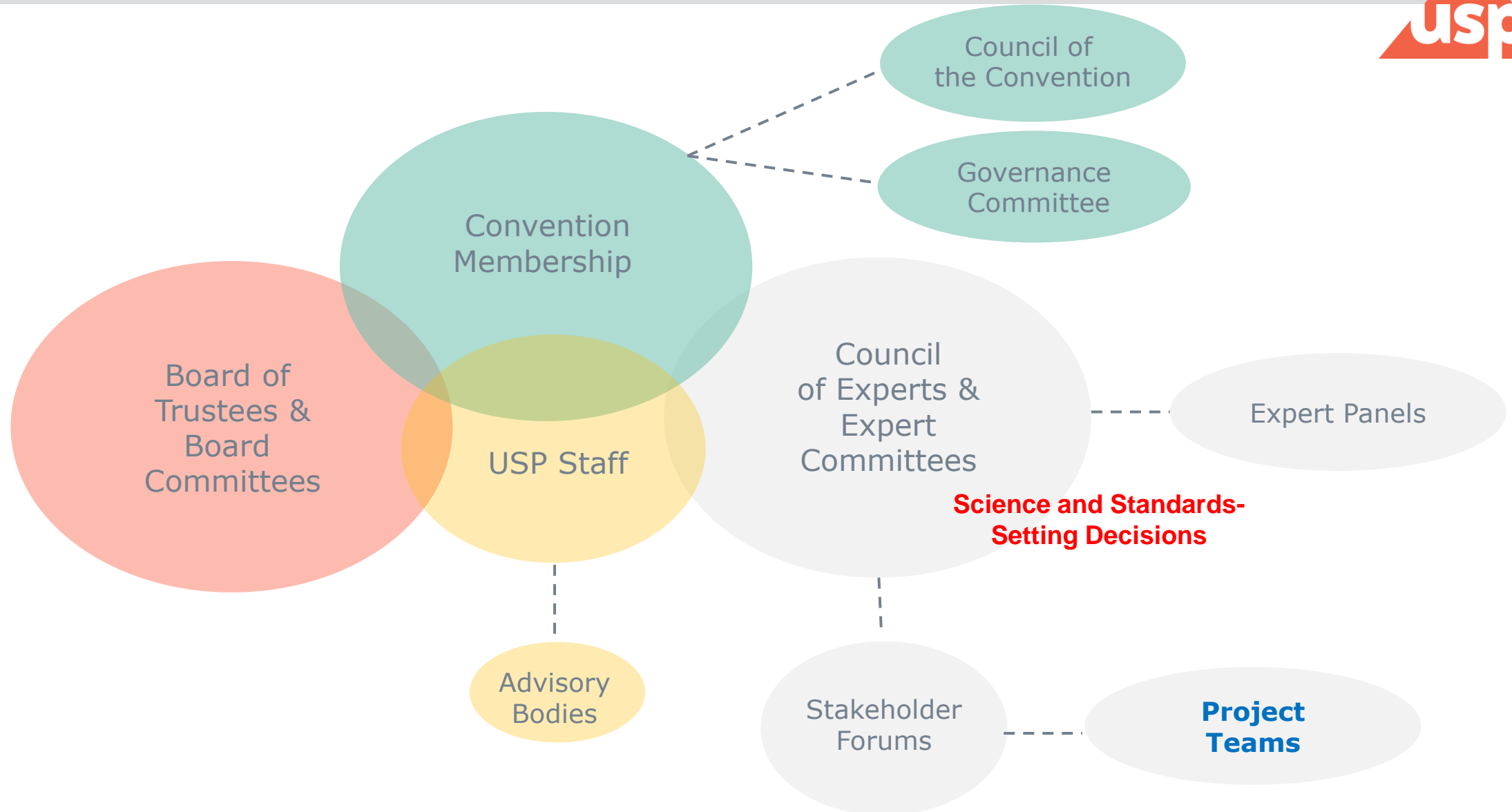


Stakeholders acknowledged the need emergence of DNA-based methods for botanical identification; however, they noted that the industry lacks enthusiasm to contribute to the development of public standards for DNA-based methods for botanical identification.

Industry is looking to USP to develop a more centralized way to approach DNA testing, thereby reducing unnecessary expansion and duplicative effort.

- A unifying approach might involve developing a set of authenticated reliable samples that can be used as a reference for positive and negative findings; or guidelines on DNA-based assays and false ID safeguards.

USP Governing and Advisory Bodies



Rules and Procedures of the Council of Experts



Advisory Stakeholder Forums and Project Teams

9.01 Formation

- Formed by CoE Chairperson

9.02 General

- Members serve as representatives of an organization, company, or service provider - advisory only

9.03 Stakeholder Forums

- Formed by CoE Chairperson to enable an exchange of information and perspectives with the ultimate goal of improving USP standards and information

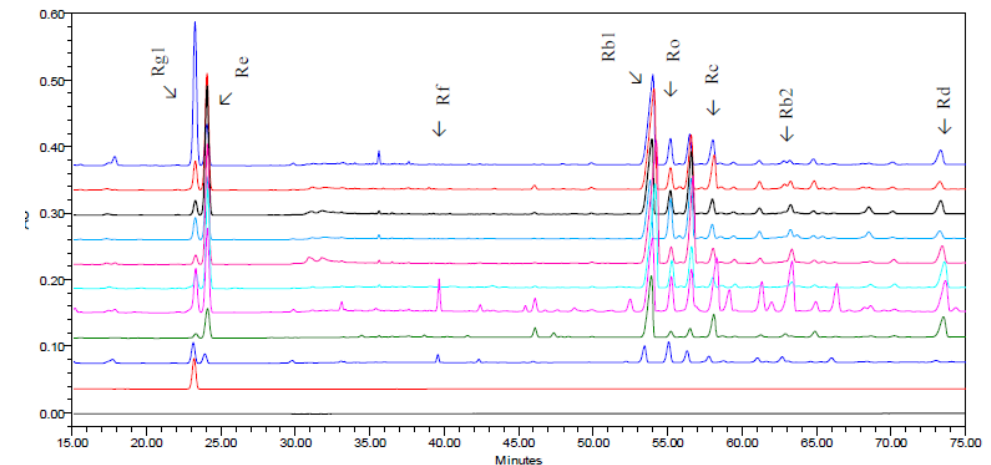
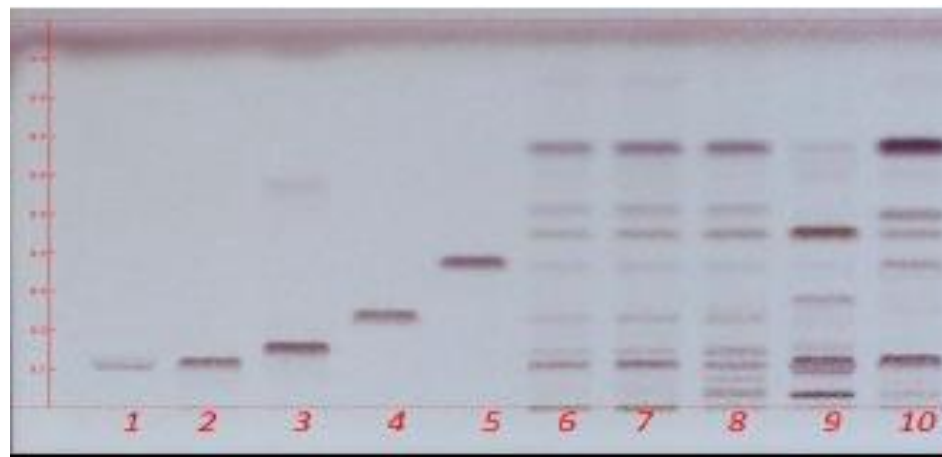
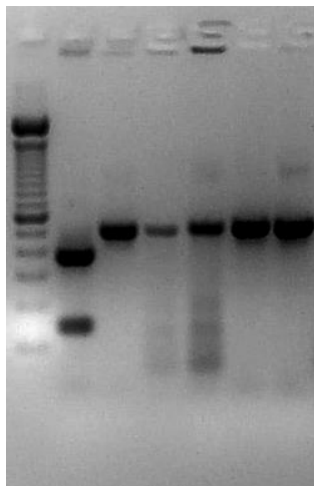
9.04 Project Teams

- Generally formed by CoE Chairperson to address a specific compendial topic (primarily process-oriented) for a particular Stakeholder Forum

Ongoing activity



- ▶ **Project Team** consisting of 6 major DS manufacturers was formed for input and materials to help USP establish an authenticated sample library for the most commonly used botanicals in dietary supplements. This helped us to procure commercially relevant samples.
- ▶ **Botanical Library** of representative samples created with commercial and vouchered authentic samples. This resource helps us in method development and in conducting orthogonal tests.
- ▶ **Proof-of-concept tests** completed using species-specific DNA methods for the ginseng species (Karen Wu, Visiting Scientist). Orthogonal tests according to current compendial HPLC methods completed and HPTLC tests underway in the CDL.



Next Steps / Actions



▶ 3 – 6 month plan:

- Marketing research to determine demand: market size, price points; format expectations; average margins for this (type of) product and competitive offerings for DNA standards across PUTs (start with DSHM, and then expand to Bio and Foods, if possible)
- USP Fellow (Ning Zhang) joined April 30, 2018.
- Set up pilot for botanical DNA method development in phase I
- Begin development of species-specific methods for 10 botanical families prioritized by the DNA Project Team.
- USP workshop on DNA Standards for Botanical Identification, August 21-22, 2018.

▶ 6 – 12 month plan:

- Form an Expert Panel; Collaboration with SMEs and collaborative testing; Introduction of methods in the monographs / GC
- Evaluate investment and timing to set up dedicated facilities and systems (phase II)
- Investigate potential partners for outsourcing bulk DNA extraction for development of reference materials
- USP work on orthogonal tests using current monograph methods based on HPLC and HPTLC
- Consideration: Industry funded activity? Develop process to address potential conflict of interest issues

▶ 12 – 18 month plan:

- Examine cross-functional application in the areas of probiotics, Biologics (residual DNA), Foods (spices; animal protein; sea food; allergens); microbiome therapies.

USP workshop on DNA Standards for Botanical Identification – August 21-22, 2018



Objective:

To provide a forum for discussion on the recent developments in the DNA technologies for botanical identification and to discuss the stakeholder needs to implement the DNA methods as a routine QA tool.

Expected outcomes:

- (a) to understand the regulatory requirements and experiences from the industry in starting to implement the new methodologies for botanical identification,
- (b) to gain an understanding of the strengths, limitations and opportunities for the harmonization of the new technologies to produce transparent quality assessment methods,
- (c) to identify the potential solutions to the challenges associated with voucher specimens and public databases, and
- (d) to identify stakeholder needs for validated DNA methods and the potential role for USP in providing testing methods and reference standards for the identification of botanical articles.



Charge: To provide industry inputs and research collaboration to help USP establish a sample repository (library) for most commonly used botanicals.

These inputs help USP:

- Develop scientifically valid uniform public standards and methodologies (USP monographs or General Chapters) to authenticate botanicals by DNA-based identification methods
- Establish public genetic standards libraries, and
- Establish appropriate Reference Standards based on botanical raw materials or nucleic acid.

Proposed List of Botanicals for DNA-based Methods Development



- American ginseng (*Panax quinquefolius*) roots
- Asian ginseng (*Panax ginseng*) roots
- Tienchi ginseng rhizome and root
- Black cohosh (*Actea racemosa*) rhizome and roots
- *Echinacea angustifolia* rhizome and root
- *Echinacea pallida* rhizome and root
- *Echinacea purpurea* aerial parts and roots
- Ginger (*Zingiber officinalis*) rhizome
- *Rhodiola rosea* root and rhizome
- Saw Palmetto (*Serenoa ripens*) ripe fruit
- Northern schisandra (*Schisandra chinensis*) fruit
- St. John's Wort (*Hypericum perforatum*) flowering tops
- Turmeric (*Curcuma longa*) rhizome
- *Valeriana officianalis* rhizome, roots and stolon

The pilot



American Ginseng (A)

Asian Ginseng (B)

Tienchi Ginseng (C)

Project Team on Botanical Library for DNA-based Identification - Updates



- ▶ The Project Team (PT) has identified botanicals of interest for building the library in order to meet the charge “to provide input and materials to help USP establish an authenticated sample library for the most commonly used botanicals in dietary supplements.”
- ▶ Cross-functional USP teams have worked together to draft internal guidelines for botanical acquisition, storage and record-keeping.
- ▶ USP received botanical samples from multiple PT members. These samples are representative of the commercially used materials for products in USP market. USP also procured additional voucher authenticated samples.
- ▶ Karen Wu, the Visiting Scientist from PC Shaw lab (Hong Kong), had developed PCR primers for species-specific methods and tested the samples for three Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*), and Tienchi ginseng (*Panax notoginseng*). This work could validate the proof of concept that species-specific methods can identify botanicals of interest with specificity, and can discriminate them from other related species.
- ▶ During the last meeting, the PT discussed the outcomes from the USP tests, and agreed with the need for additional collaborative testing with other closely related species to test potential false positive and false negative outcomes.
- ▶ USP Compendial Development Lab (CDL) tested the ginseng family samples for identification based on compendial HPLC / HPTLC methods.
- ▶ The PT is supportive of USP activities to expand the General Chapter <563> Identification of Articles of Botanical Origin to include the widely used DNA methods for botanical identification.