Method Validation Experiments for DNA Barcoding

A Proposal

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How do we figure this out?

TECHNICAL COMMUNICATIONS

Probability of Identification: A Statistical Model for the Validation of Qualitative Botanical Identification Methods

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Botanical Identification Method

- Any qualitative method that reliability identifies a botanical material and returns a binary result
  - 0 = “not identified”
  - 1 = “identified”
- Based on:
  - number of replicates
  - Probability of Identification (POI)
- Needs to show:
  - inclusivity, exclusivity, probability of identification, robustness, reproducibility, repeatability, “other”

(LaBudde & Harnley, 2012)

Proof of Identity

- Statistical model to determine confidence in the qualitative method
- Depends on the concentration of target plant
- Question: how does the method make the transition from the positive to the negative response?
- Method Performance Requirements (MPR)
  - Specify the botanical material (inclusivity)
  - Specify the non-target materials (exclusivity)
  - Physical form of the materials
  - Minimum concentration
  - Define categories of “specific superior” and “specific inferior” test materials

Ideal Goal

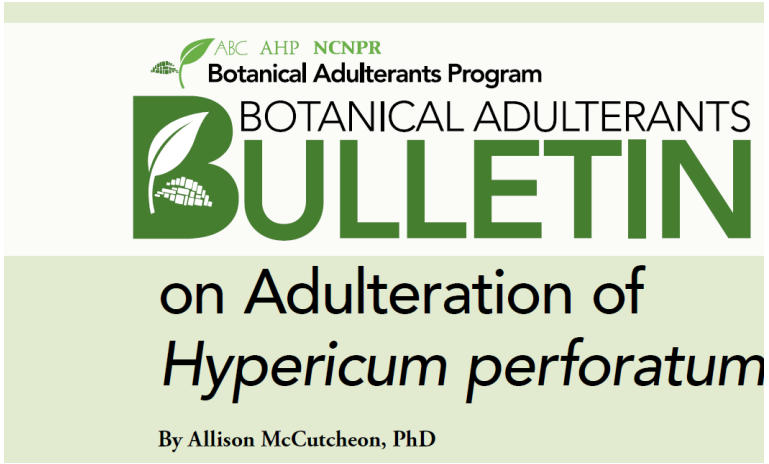
- Discriminate with a specified degree of confidence between the included and excluded

Inclusivity

- Ability of a BIM to correctly identify variants of the target material that meet the identity specification.
- Inclusivity Sampling Frame (ISF) – a list of practically obtainable botanical materials that are expected to give a positive result
  - Species
  - Subspecies
  - Cultivars
  - Growing location
  - Growing conditions
  - Post-harvest processing

What does the experiment look like?

- For each BIM, the MPR must provide a list of all necessary botanical variants that should provide a positive identification
- Case Study – St. John's wort



Inclusion:

- *Hypericum perforatum* L.

Exclusion:

- Chinese SJW (*Hypericum perforatum* subsp. chinense)
- Hybrids
- *Hypericum barbatum*
- *Hypericum hirsutum*
- *Hypericum montanum*
- *Hypericum elegans*
- *Hypericum patulatum*
- *Hypericum tetrapterum*
- 64 *Hypericum* species found in China

Study Design

= 160 samples +++

Number	Sample Identification
Inclusion Panel	
60	*Hypericum perforatum* - Wildtype
10	*Hypericum perforatum* – CV Anthos
10	*Hypericum perforatum* – CV Helos
10	*Hypericum perforatum* – CV Topas
Exclusion Panel	
10	*Hypericum perforatum* subsp. chinense
10	*Hypericum barbatum*
10	*Hypericum hirsutum*
10	*Hypericum montanum*
10	*Hypericum elegans*
10	*Hypericum patulatum*
10	*Hypericum tetrapterum*
???	64 *Hypericum* species found in China
Include or Exclude??	
???	Hybrids

Study Design (cont'd)

Number	Sample Identification
	Specific Superior Test Material (SSTM)
420	*Hypericum perforatum* (98%) + adulterant (2%) (60 per test x 7 likely adulterants)
	Specific Inferior Test Material (SITM)
60	*Hypericum perforatum* (90%) + *Hypericum perforatum* subsp. chinense (10%)
60	*Hypericum perforatum* (90%) + *Hypericum barbatum* (10%)
60	*Hypericum perforatum* (90%) + *Hypericum hirsutum* (10%)
60	*Hypericum perforatum* (90%) + *Hypericum montanum* (10%)
60	*Hypericum perforatum* (90%) + *Hypericum elegans* (10%)
60	*Hypericum perforatum* (90%) + *Hypericum patulatum* (10%)
60	*Hypericum perforatum* (90%) + *Hypericum tetrapterum* (10%)
???	64 *Hypericum* species found in China (?????)
	Include or Exclude??
???	Hybrids (?????)

= 840 samples +++

Interpreting the data

Table 2. Example performance requirements

Requirement	SSTM, %	Measure	Limit	No. of replicates to be tested	No. of failures allowed[a]
POI	100	95% 1-sided LCL	0.90 (FNF<0.10)	60	2
POI	0	95% 1-sided UCL	0.10 (FPF<0.10)	60	2

[a] In each case, no more than two failures are allowed.

Table 4. Observed SLV results for example BIM

SSTM, %	No. of test portions	No. identified	No. not identified	POI
0.0	60	1	59	0.0167
33.3	60	7	53	0.1167
66.7	60	27	33	0.4500
100.0	60	60	0	1.0000

Still need… SLV, Co-Lab Trial, chemical tests

Table 7. Collaborative study results for 0% SSTM concentration

AOAC Binary Data Interlaboratory Study Workbook Study Reported Values					Version: 2.2
	Sample ID 0% SSTM				
Sequence	Item	Symbol	Value	Approximately 95% LCL[a]	Approximately 95% UCL[b]
1	Total number of laboratories	p	10		
2	Total number of replicates	Sum(n(L))	120		
3	Overall mean of all data (grand mean)	LPOI or LPOD	0.0083	0.0015	0.0457
4	Repeatability SD	s(r)	0.0913	0.0807	0.1713
5	Among-laboratories SD	s(L)	0.0000	0.0000	0.0402
6	Homogeneity test of laboratory PODs	P-value	0.4303		
7	Reproducibility SD	s(R)	0.0913	0.0814	0.1064
8	Intraclass correlation coefficient for repeatability	I(r)	1.0000	0.8335	1.0000

[a] LCL = Lower confidence level.

[b] UCL = Upper confidence level.

Also the following admixture of dyes have been reported as co-occurring SJW adulterants: E123 Amaranth (FD&C Red #2), E133 Brilliant Blue (FD&C Blue #1), E110 Sunset Yellow (FD&C Yellow #6), and E102 Tartrazine (FD&C Yellow #5).[25]