104年赴美參加不法藥物摻偽及食品假冒之檢驗技術研討會及參訪美國食品藥物管理局出國分享報告

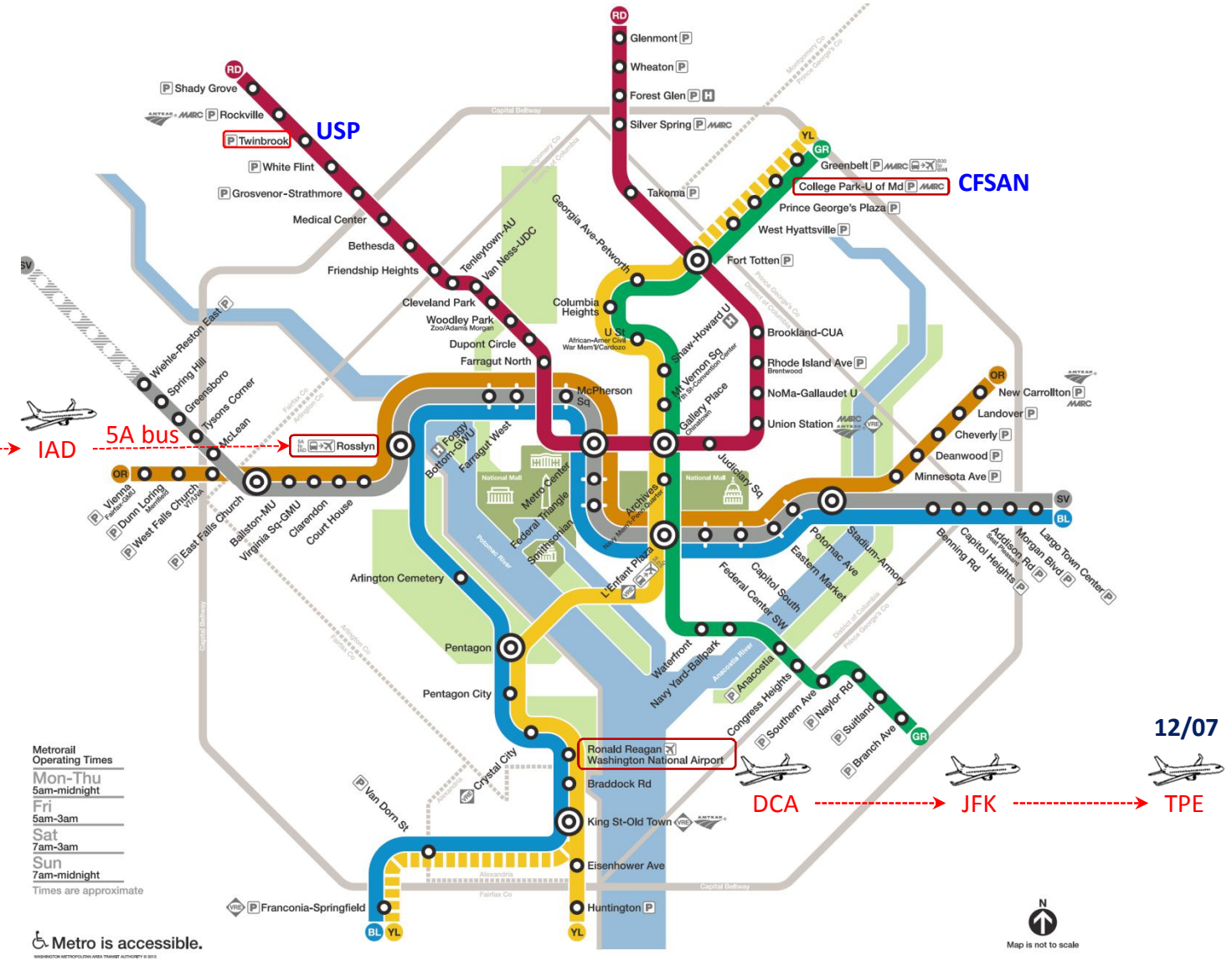
報告日期：105年02月24日

報告人：方俊仁技士、黃昱裴技士、蔡佳芬科長

出國地點：1. 美國藥典委員會(USP)
2. 美國食品藥物管理局
食品安全暨應用營養中心(FDA CFSAN)

11/30

TPE ⇢ LAX ⇢ IAD 5A bus ⇢



DCA ⇢ JFK ⇢ TPE

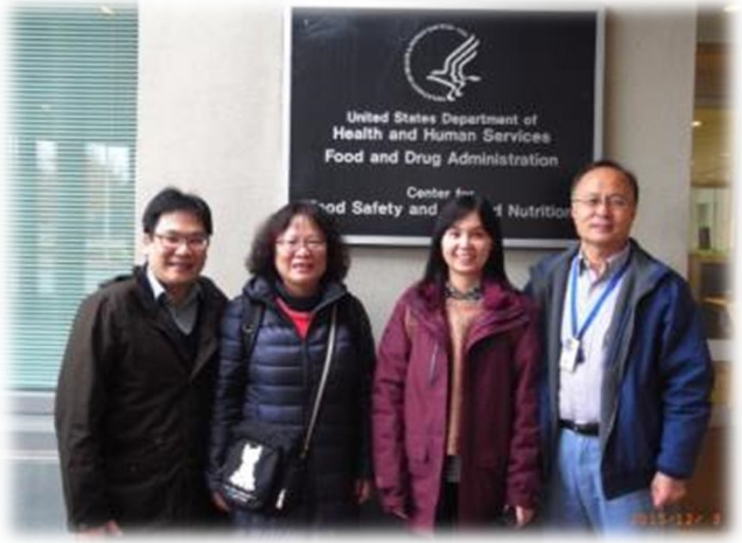
12/07

參訪美國食品藥物管理局

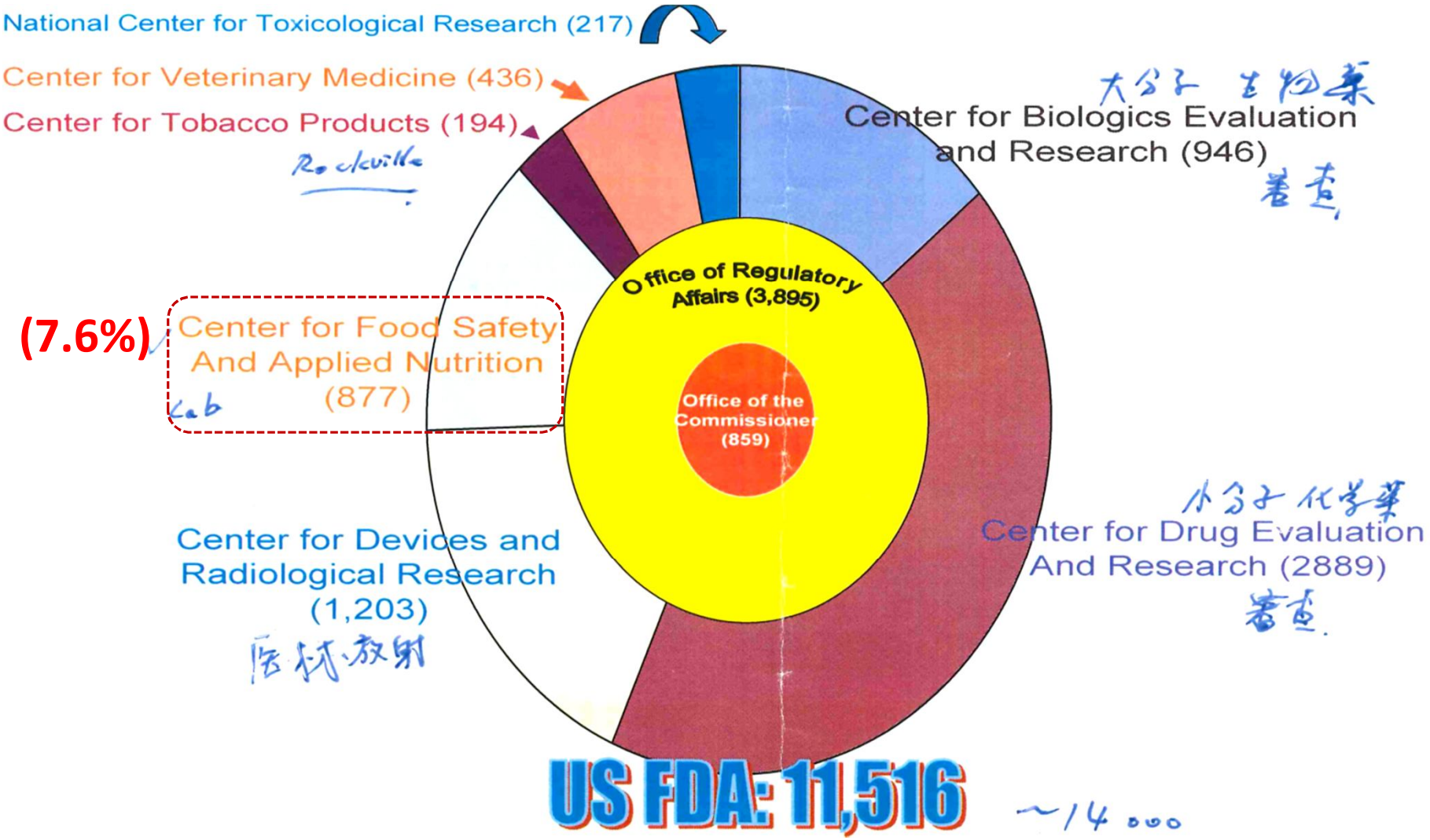
食品安全暨應用營養中心

美國食品藥物管理署

- 局長辦公室(OC)
- 監管事務辦公室(ORA)
- 藥品查驗暨研究中心(CDER)
- 生物製劑查驗暨研究中心(CBER)
- 醫療器械暨放射健康中心(CDRH)
- **食品安全暨應用營養中心(CFSAN)**
- 動物用藥中心(CVM)
- 菸草產品中心(CTP)
- 國家毒理研究中心(NCTR)



人力資源配置圖



圖表資料係Dr. Perry G. Wang提供

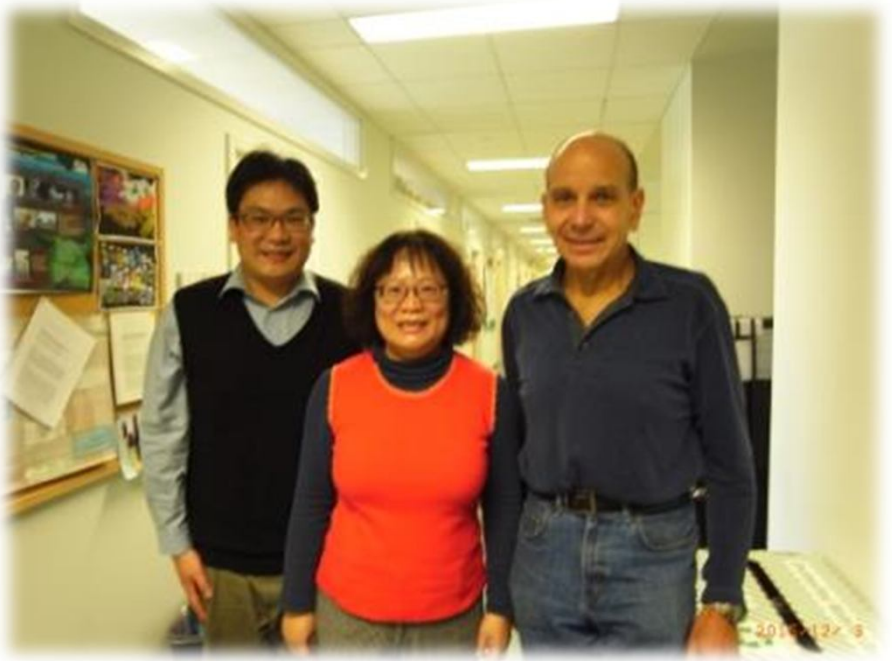
Dr. Jon W. Wong (左)介紹實驗室

U.S. FOOD AND DRUG ADMINISTRATION
Center for Food Safety and Applied Nutrition

Jon W. Wong, Ph.D.
Research Chemist
Bioanalytical Methods Branch
Division of Bioanalytical Chemistry

Office of Regulatory Science
5100 Paint Branch Parkway
HFS-706
College Park, MD 20470-3835

Telephone: (240) 402-2172
Fax: (301) 436-2332
E-mail: jon.wong@fda.hhs.gov



與Dr. Alexander J. Krynistky合影(右)
蔡佳芬科長(中)，方俊仁技士(左)

U.S. FOOD AND DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

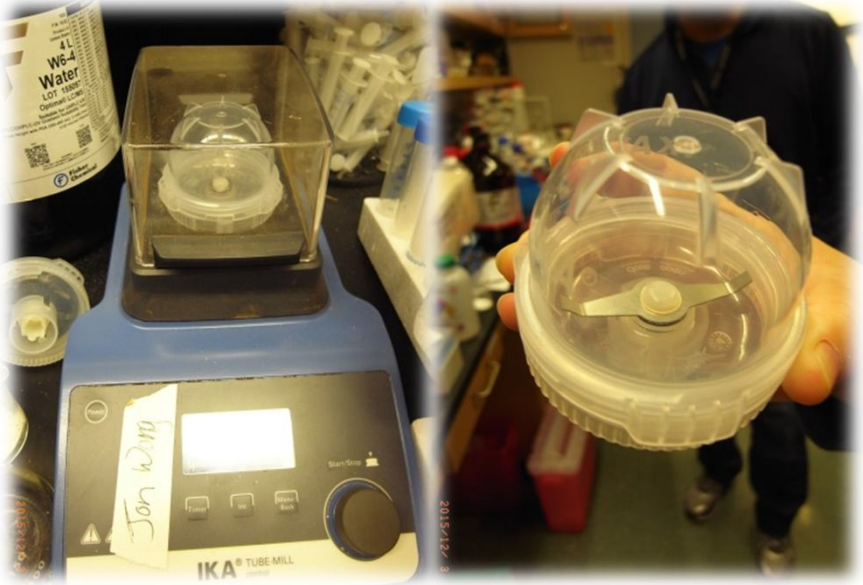
Alexander J. Krynistky
Chief, Bioanalytical Methods Branch
Division of Bioanalytical Chemistry

Office of Regulatory Science (HFS-717)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Telephone: (240) 402-2098
BlackBerry: (301) 204-4296
Fax: (301) 436-2332
E-mail: alex.krynitsky@fda.hhs.gov

Pesticides screening

Sample prep. and store

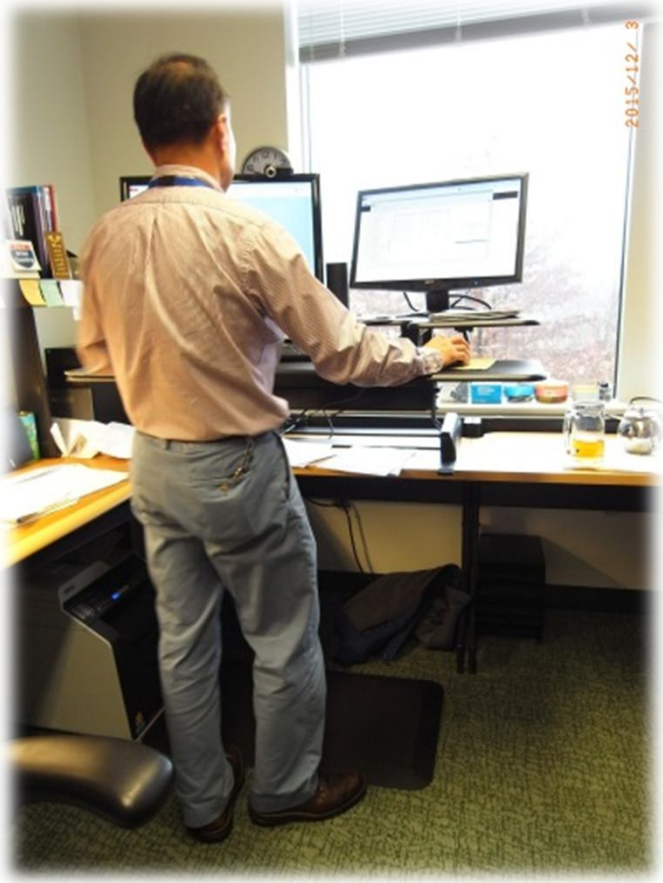


Dioxin analysis

左上: Thermo Q Exactive Plus LC-MS/MS

左下: Thermo Q Exactive GC-MS/MS

右上: IKA Tube Mill



U.S. Food and Drug Administration
Center for Food Safety & Applied Nutrition

Perry G. Wang, Ph.D.
Research Chemist
Division of Bioanalytical Chemistry

5100 Paint Branch Parkway
HFS-717, Room: 4E-019
College Park, MD 20740

Telephone: (240) 402-1609
Fax: (301) 436-2694
E-mail: perry.wang@fda.hhs.gov

右: 與Dr. Perry G. Wang合影
左: 可升降式辦公檯面外觀

參加「不法藥物摻偽及食品假冒之檢驗技術研討會」

USP's Food Fraud Mitigation Guidance as Tool for Industry

Jeff Moore, Ph.D.
Director, Food Standards
USP
Rockville, Maryland

- Tools for Food Fraud Mitigation

1. USP Food Fraud Database (鑑往知來)
2. **USP Food Fraud Mitigation Guidance (弱點評估)**
3. USP FCC-Identity Standards (身分識別)

Food Fraud Mitigation Guidance Approach

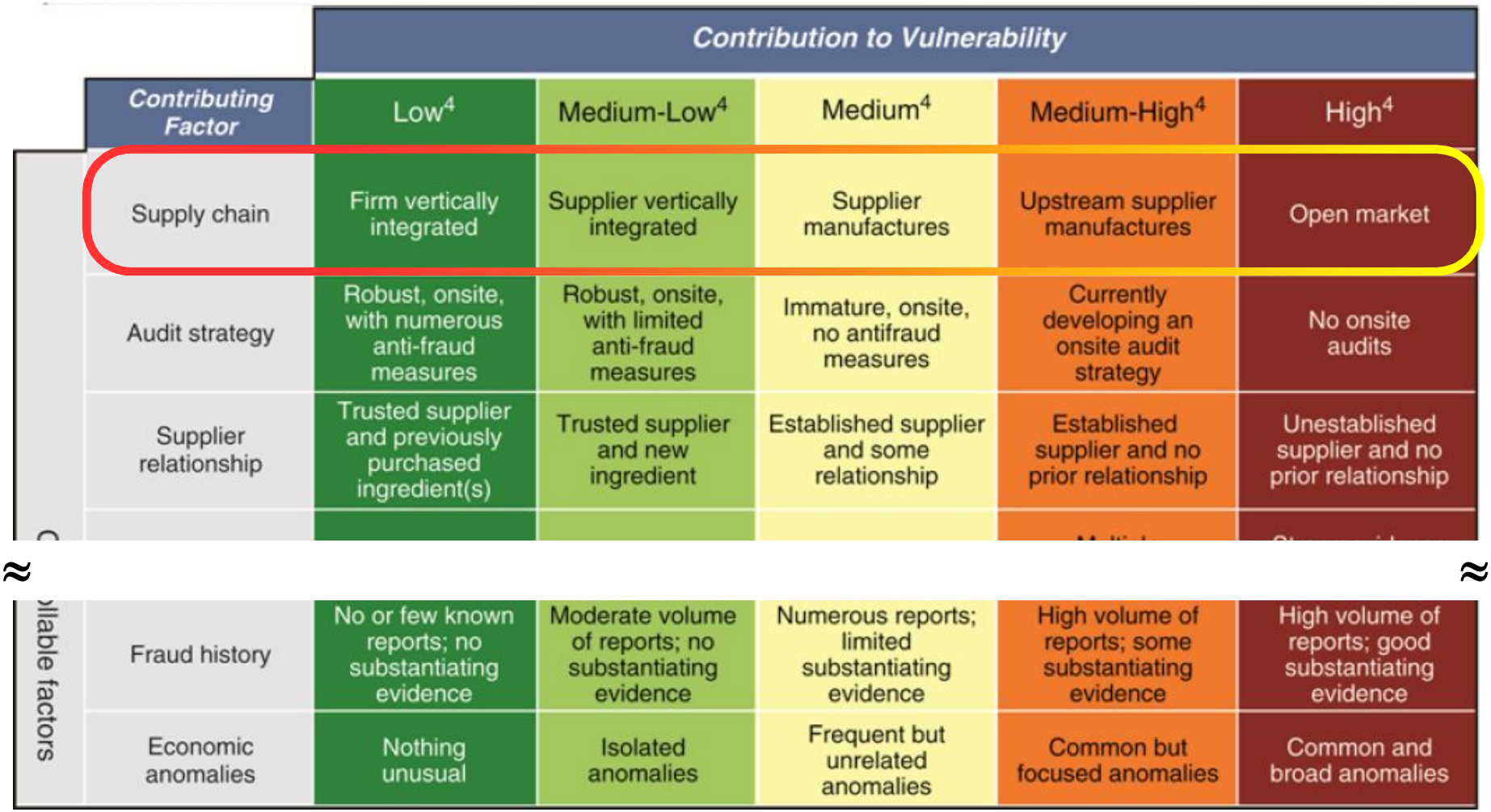
Ingredient by ingredient…..

Vulnerabilities characterization

1. Contributing factors assessment
2. Impacts assessment
3. Combining 1 & 2

4. Mitigation strategy development

Step.1 Contributing Factors Assessment



	Contributing Factor	Low[4]	Medium-Low[4]	Medium[4]	Medium-High[4]	High[4]
		Contribution to Vulnerability				
	Supply chain	Firm vertically integrated	Supplier vertically integrated	Supplier manufactures	Upstream supplier manufactures	Open market
	Audit strategy	Robust, onsite, with numerous anti-fraud measures	Robust, onsite, with limited anti-fraud measures	Immature, onsite, no antifraud measures	Currently developing an onsite audit strategy	No onsite audits
	Supplier relationship	Trusted supplier and previously purchased ingredient(s)	Trusted supplier and new ingredient	Established supplier and some relationship	Established supplier and no prior relationship	Unestablished supplier and no prior relationship
≈						≈
llable factors	Fraud history	No or few known reports; no substantiating evidence	Moderate volume of reports; no substantiating evidence	Numerous reports; limited substantiating evidence	High volume of reports; some substantiating evidence	High volume of reports; good substantiating evidence
	Economic anomalies	Nothing unusual	Isolated anomalies	Frequent but unrelated anomalies	Common but focused anomalies	Common and broad anomalies

raw material > ingredient > product

Step.2 Potential Impacts Assessment

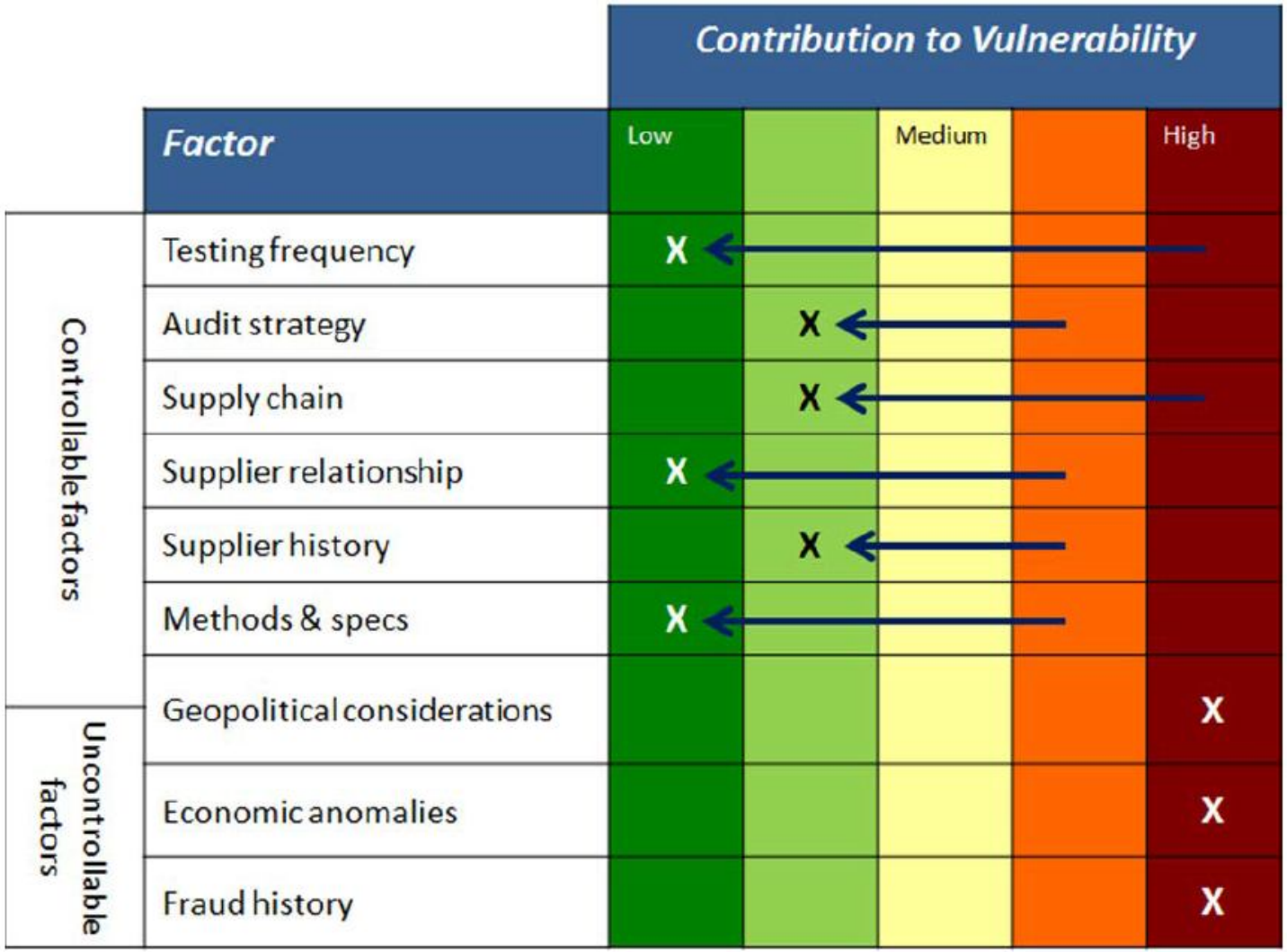
	Low		Moderate		High
Food Safety	Food grade-known safe	Food grade-No known risks	Food grade-known sub-population risks	Non-food/ non-food grade-unknown risks	Non-food/non-food grade-known risks
Economic Impact	No significant balance sheet impact		Operational Risk		Enterprise risk
Potential Multipliers					
Focused Consumption	No focused consumption	Temporally focused	Low level	Potential target populations	At-risk populations
Nutritional Sufficiency	No sufficiency impacts		Important micro-nutrient food	Core food for a sub-population	Primary/critical sub-population food
Public Confidence	Specific food	Specific commodity	Industry sector	Industry wide	Authorities & industry

Step.3 Overall Vulnerabilities Characterization

			Contributing Factors (Composite of Step 1)				
			1	2	3	4 Step 1	5
Potential Impact (Composite of Step 2)			Low	Medium-Low	Medium	Medium-High	High
	A	Low Economic	New controls optional	New controls optional	New controls optional	New controls optional	New controls should be considered
	B	Moderate Economic	New controls optional	New controls should be considered	New controls should be considered	New controls should be considered	New controls strongly recommended
	C	Low Public Health/High Economic	New controls optional	New controls should be considered	New controls should be considered	New controls strongly recommended	New controls strongly recommended
	D	Moderate Public Health/High Economic	New controls optional	New controls should be considered	New controls strongly recommended	New controls strongly recommended	New controls strongly recommended
	E	High Public Health/High Economic	New controls optional	New controls strongly recommended	New controls strongly recommended	New controls strongly recommended	New controls strongly recommended

Step 2

Step.4 Mitigation Strategy Development

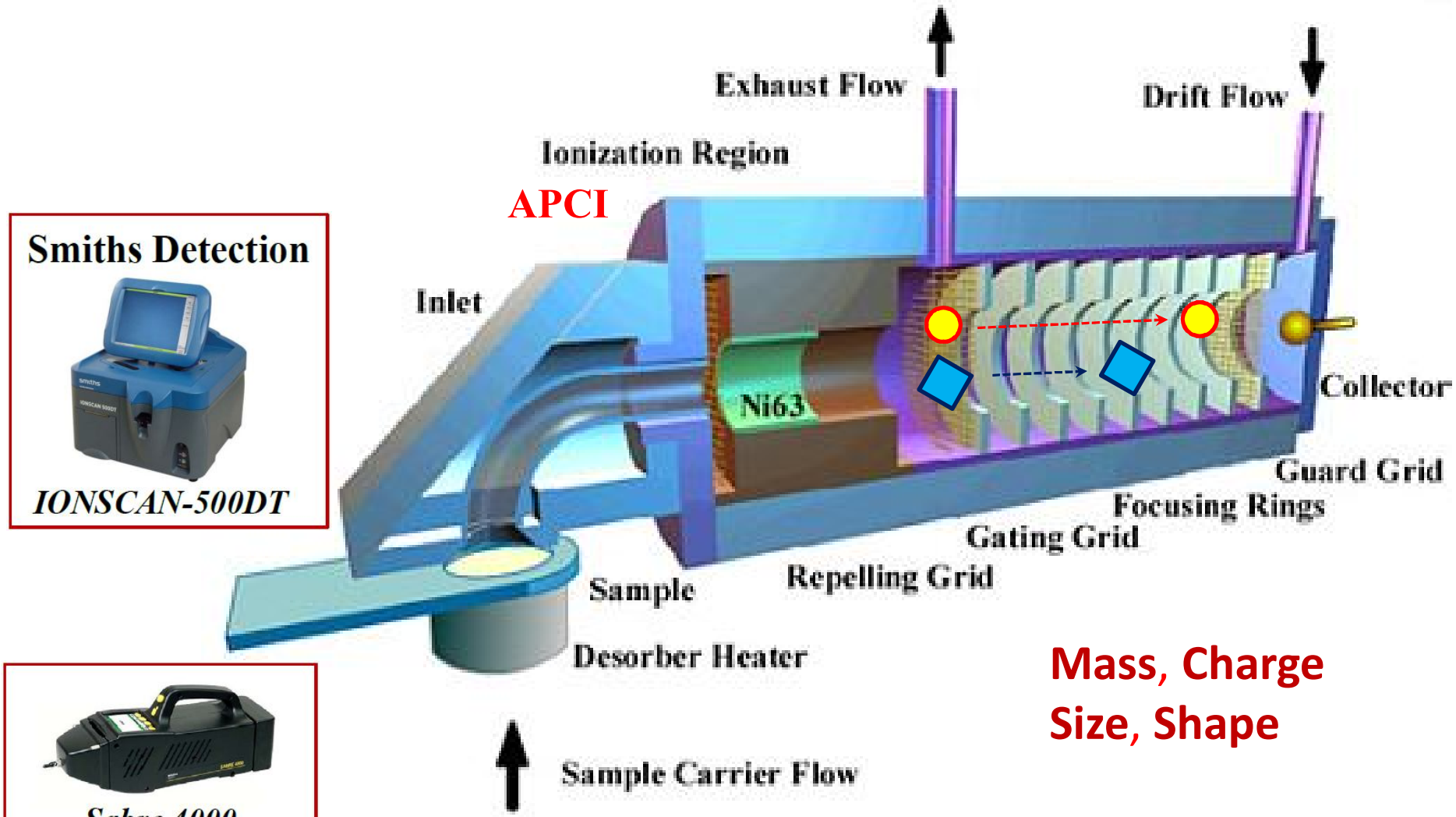


Field Investigation of DS Adulteration

Connie Gryniewicz -Ruzicka, Ph.D.
Division of Pharmaceutical Analysis
US Food and Drug Administration
Saint Louis, Missouri

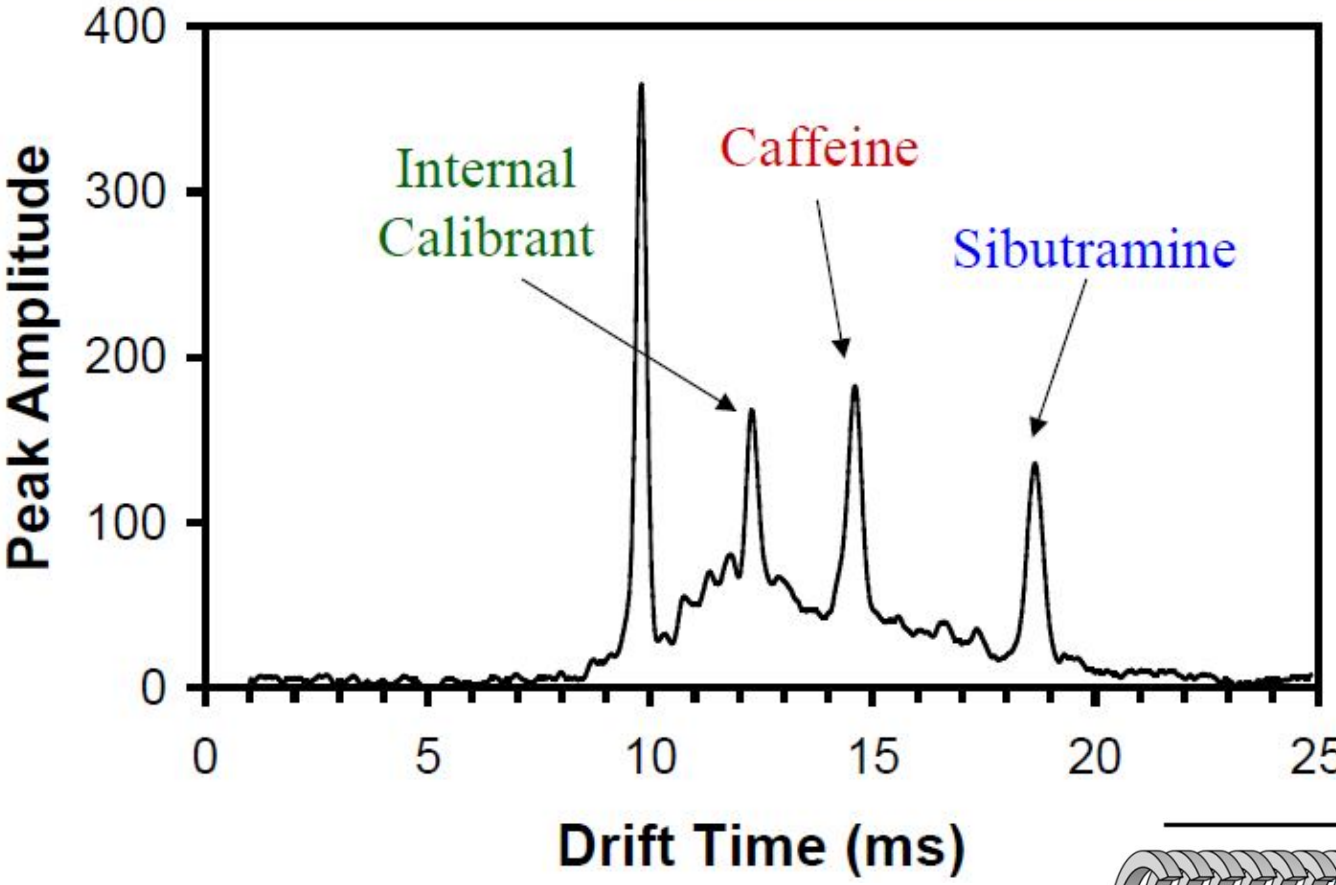
- Rapid Screening
- Field Deployable Analytical Methods
  - **Ion Mobility Spectrometers (IMS)**
    - **Rapid, portable, easy**
    - **Sensitivity, specificity**
    - **User friendly ……**

Ion Mobility Spectrometer

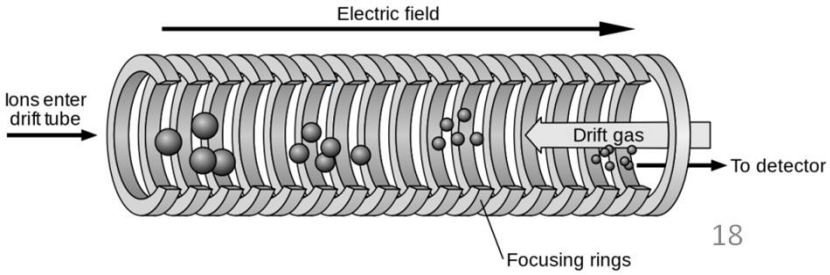


https://www.smithsdetection.com/index.php?option=com_k2&view=item&layout=item&id=40&Itemid=638

Ion Mobility Spectrum



$$K = \frac{v_d}{E} = \frac{L^2}{V * t_d}$$



K: mobility, L: length of drift tube

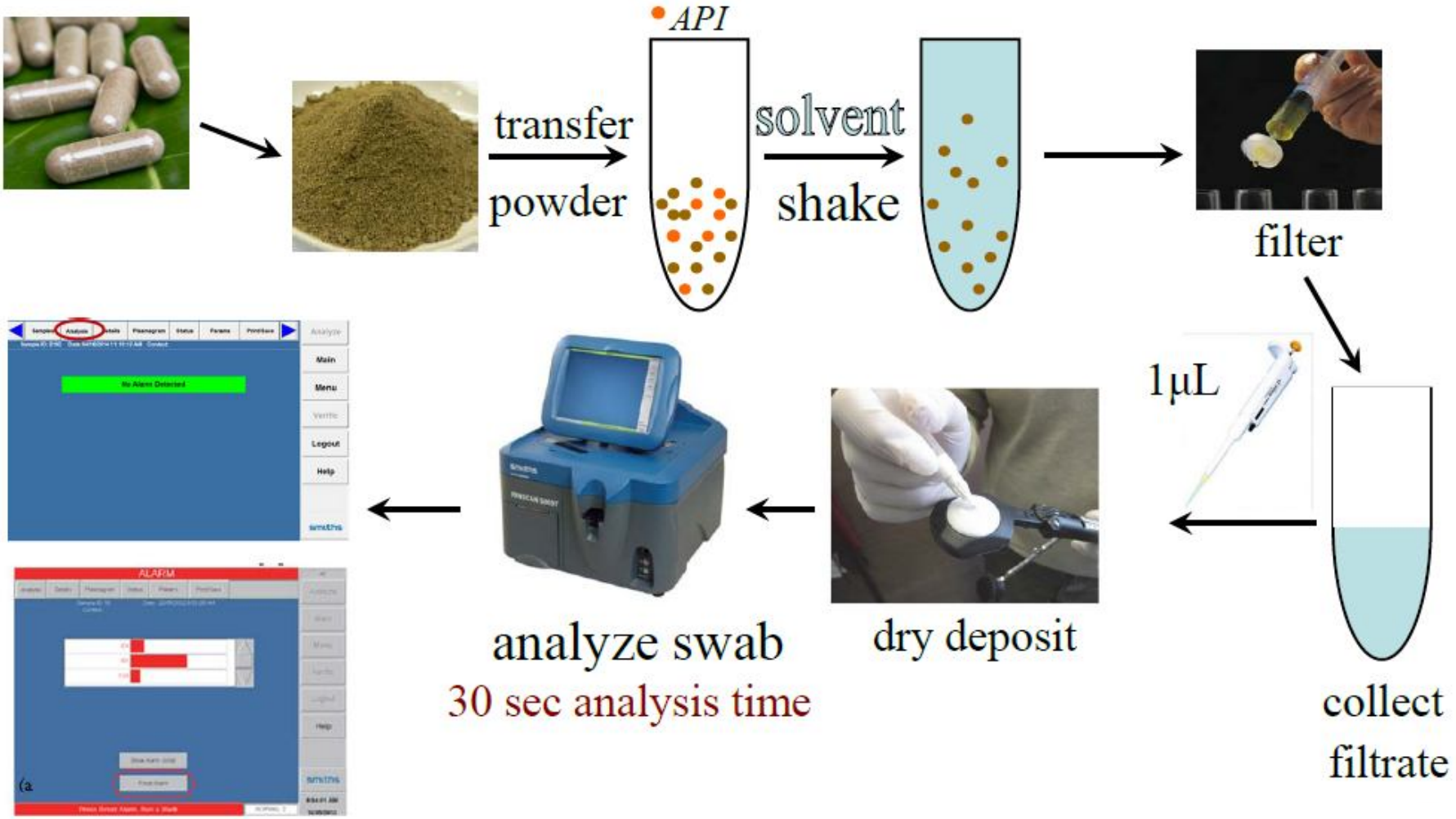
V: electrical potential difference, t_d: drift time

Smiths Detection IONSCAN 500DT

https://youtu.be/r9t5tBIwQP0

https://www.smithsdetection.com/index.php?option=com_k2&view=item&id=115:ionscan-500dt&Itemid=1427#.VsVkW7kw-rA

Sample Preparation



Weight Loss Compounds

Instrument	IONSCAN-500DT			Sabre 4000		
Type	Benchtop			Portable		
Calibrant (Positive)	Nicotinamide			Nicotinamide		
Substrates (Swabs)	Nomex			Shark Fin		
Compound	**K_o ($cm^2/V•s$)**	**FWHM**	**LOD (ng)**	**K_o ($cm^2/V•s$)**	**FWHM**	**LOD (ng)**
1,3-dimethylamylamine	1.6986	275	1	1.6458	330	5
Desmethyl sibutramine	1.1969	390	1	1.1898	440	1
Didesmethyl sibutramine	1.2215	375	0.5	1.2100	420	1
Fenfluramine	1.3691	340	0.1	1.3541	360	1
Fluoxetine	1.1284	380	1	1.1197	410	2
Lorcaserin	1.4288	355	2	1.407	350	5
Orlistat	Not Detected			1.2233	370	20
Phenolphthalein*	1.1325	395	20	1.1369	420	500
Phentermine	1.5979	280	0.1	1.5645	350	1
Phenytoin	1.5315	290	20	1.2415	450	500
Rimonabant	0.8722	600	15	0.8765	550	500
Sertraline	1.1727	410	0.5	1.1732	400	5
Sibutramine*	1.1722	400	1	1.1706	430	2

*Most common adulterants detected in weight loss products.

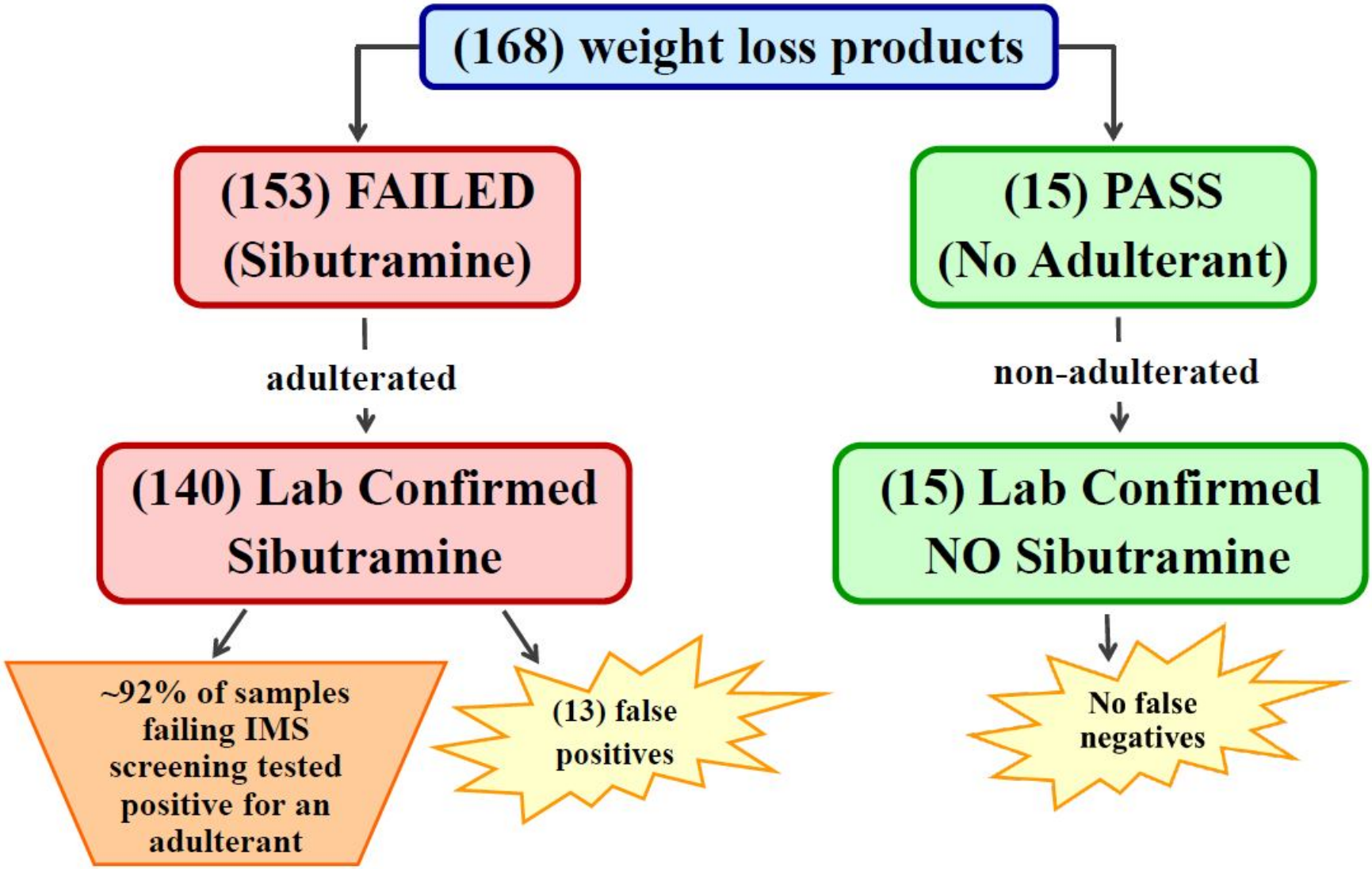
Male Enhancement Compounds

Compound	K_o ($cm^2/V \cdot s$)	FWHM (du)	LOD (ng)
Acetildenafil	0.8693	379	10
Acetyl Vardenafil	0.8201	590	5
Amino Tadalafil	1.0140	443	25
Avanafil	0.8269	576	7.5
ChloroPreTadalafil	0.9464	469	5
Homosildenafil	0.8556	521	5
Hydroxy Acetildenafil	0.8491	373	5
Hydroxyhomosildenafil	0.8350	504	10
Hydroxythiohomosildenafil	0.8236	548	10
Methisosildenafil	0.8561	491	2
N-Desethyl Vardenafil	0.8675	486	5
N-Desmethyl Sildenafil	0.8839	500	5
Nor-Acetildenafil	0.8847	492	5

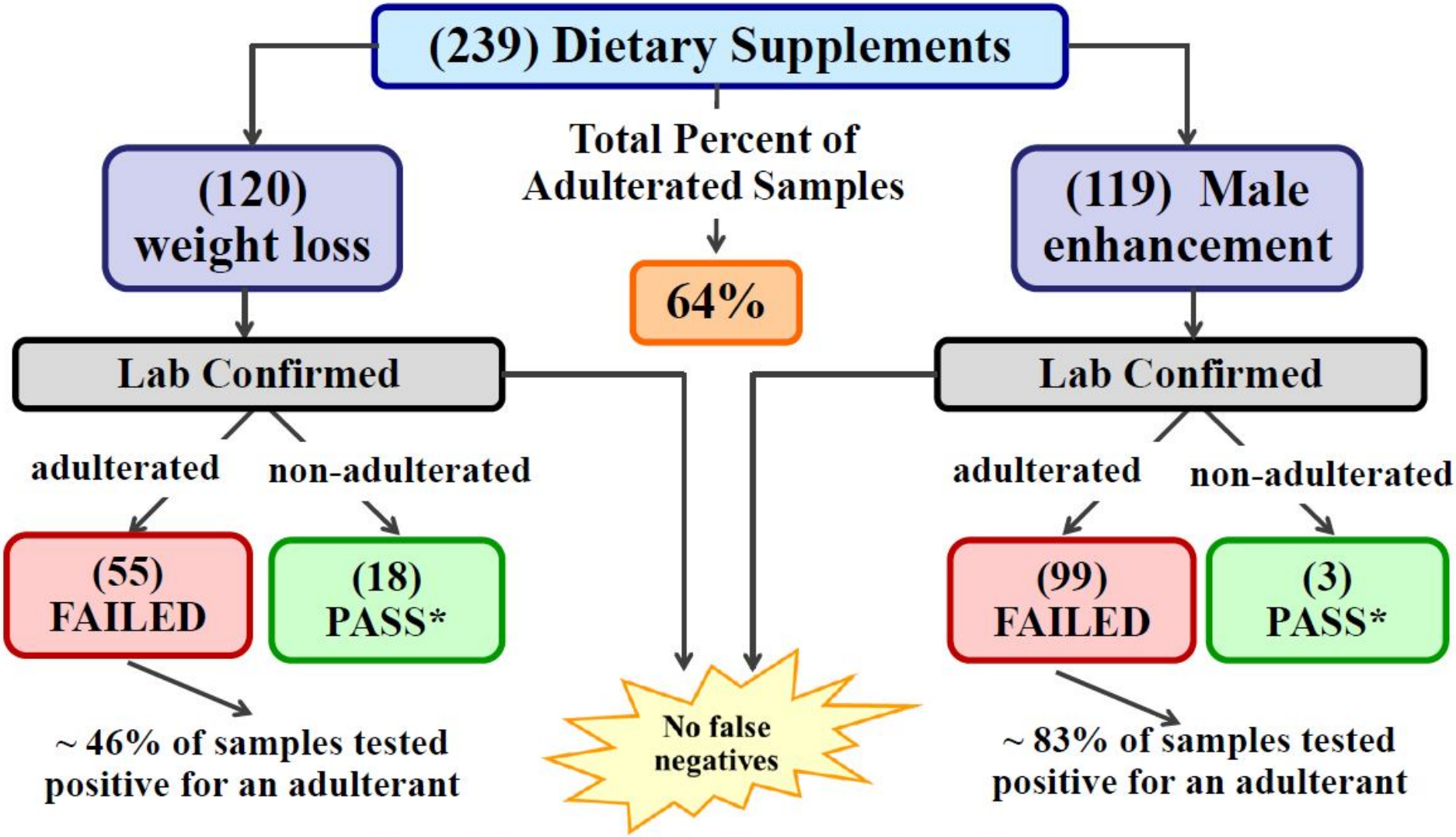
Compound	K_o ($cm^2/V \cdot s$)	FWHM (du)	LOD (ng)
Norneosildenafil	0.8640	505	2
Piperiacetildenafil	0.8590	511	5
Piperidenafil	0.8638	511	5
Sildenafil*	0.8719	479	10
Tadalafil*	1.0068 1.2831	419 343	10
Thiohomosildenafil	0.8510	527	5
Thiomethisosildenafil	0.8505	541	5
Thiosildenafil*	0.8695	631	5
Udenafil	0.8153	548	5
Vardenafil	0.8197	500	7.5
Xanthroanthrafil	1.2179	801	10

*Most common adulterants detected in male enhancement products

Portable IMS – Field Deployment Pilot Study



Benchtop IMS – Field Deployment



*value includes 10th negative samples sent to the lab and false positives

Adulteration of Food with Illegal Dyes

Thomas Tarantelli
Senior Food Chemist
NYS Dept.
Agriculture and
Markets
Albany, New York

LC/MS/MS color screen 36 compounds

(Basic dyes, Acid dyes, Solvent dyes, Pigments)

Analyte	RT	T 1	T 2
Alizarin	2.72	241>156.8	241>185
Auramine O	1.25	268>121.9	268>146.8
Basic Blue 3	1.25	324>236	324>280
Basic Red 46	1.21	321.1>224.0	321.1>196.0
Basic Yellow 13	1.25	307>169.9	307>292.1
Basic Yellow 28	1.25	322>135.9	322>160
Brilliant Green	2.23	385.1>297	385.1>341
Chrysoidin G	1.22	213>76.9	213>120.9
Citrus Red 2	8.07	309>151.8	309>137.8
Crystal Violet	1.80	372>340	372>356.2
Dimethyl Yellow	6.47	226>76.9	226>133.8
Disperse Orange 11	3.81	238>165	238>194.9
Disperse Red 9	5.41	238.1>165	238.1>194.9
Fast Garnet GBC	5.05	226>90.8	226>105.8
Malachite Green	1.39	329.1>208.1	329.1>313.1
Metanil Yellow	4.03	354>109	354>169
Oil Orange SS	9.05	263>106.8	263>155.9
Orange II	3.69	329>127.9	329>155.9
Para Red	7.18	293.9>127.9	293.9>155.9
Pararosaniline	1.13	288.0>167.8	288.0>195
Quinoline Yellow SS	3.68	273.9>76.9	273.9>104.8
Rhodamine 6G	1.79	443>341	443>415.1
Rhodamine B	1.99	443>355	443>399
Safranin O	1.27	315>237	315>299
Sudan Black	9.80	457>194	457>211
Sudan I	8.09	248.8>92.8	248.8>127.8
Sudan II	9.84	277>120.8	277>167.1
Sudan III	10.50	249.2>92.6	249.2>155.8
Sudan IV	11.49	381.2>276.1	381.2>224.1
Sudan Orange G	4.29	215>92.8	215>121.8
Sudan Red 7B	11.06	378>77	378>273
Sudan Red B	11.34	381.2>90.8	381.2>105.8
Sudan Red G	7.93	279>107.9	279>122.9
Toluidine Red	7.76	308>105.9	308>127.9
Yellow AB	7.17	247.9>92.9	247.9>231
Yellow OB	8.07	262>106.9	262>156.9

Honey Adulteration

Lutz Elflein, Ph.D.
Intertek Food Services
Bremen, Germany

Untargeted Approach:

- Isotopic Screening (mostly ^{13}C)
- Spectroscopic Screening (FTIR, NIR, Fluorescence, Raman)
- Nuclear Magenetic Resonance (SNIF-NMR; ^{1}H-NMR Profiling)
- High Res. MS-Profiling (GC-TOF; LC-HRMS)

Advantage:

- Independent of type of adulteration

Disadavantages:

- Authentic reference databases required
- LODs vary with type of adulteration
- Sophisticated, high effort

Targeted Approach:

- Specific Marker Methods (Marker: substance specfic for the added syrup or the production process
- GC-MS, LC-MS, LC, ICP-MS, Enzymatic Tests

Advantage:

- Fast and very sensitive

Disadavantages:

- Detects only one type of adulteration
- LOD depends on compound concentration

Take a break

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

- Francoise Dorcier
- Criminal Intelligence Officer, INTERPOL, France
- She is in charge of Operation OPSON targeting counterfeit and/or substandard food and beverages.

Overview of INTERPOL

Food fraud and DSF: scope and impact

INTERPOL's responses

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

Overview of INTERPOL

- Founded in 1923
- 190 member countries

INTERPOL's core functions

- Secure global police communications network
- Tools permitting the identification of crime and criminals
- Around-the-clock operational support to law enforcement worldwide
- Capacity building
- Business continuity and sustainability
- Institutional and legal support

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

TYPES OF INFRINGEMENT ON FOOD FRAUD

偽造

Counterfeiting

Counterfeit branded alcohol

冒充

Substitution

Horsemeat scandal

Substitution of beef by horsemeat

摻假

Substitution/Adulteration

Olive oil substitued or diluted with lower-cost oil (nut, sunflower, palm ...)

標示不實

Substitution/Mislabelling

Substitution of expensive fish with cheaper species

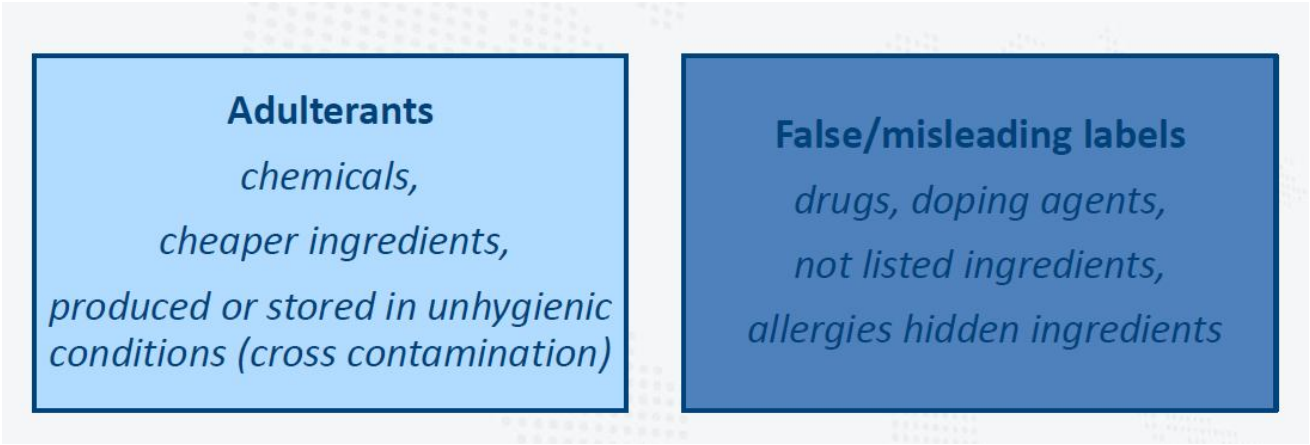
Labelled as wild fish but are farmed fish

Modification of seafood product

Increasing weight (over icing), use of unapproved additives

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

Fraud on Dietary Supplements



What are the challenges to fight against DSF?

- May contain potential dangerous ingredients difficult to detect
- Are often bought on internet
- Low awareness among consumers

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

INTERPOL's responses

Operation OPSON			
2011	2012	2013	2014/2015
10 countries	29 countries	31 countries	47 countries

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

OPSON IV

Tons
11,591.76

Liters
814,563.19

Pieces:
1,233,911

Products Seized or withdrawn from markets

Italy-Spain: Frozen fish sold as fresh fish

Italy: Illicit production of mozarella

Rwanda: counterfeit beer

INTERPOL's Initiatives to Combat Fraud in Foods and Dietary Supplements

Operation Pangea VIII: 9 – 16 June 2015

(19 May – 16 June 2015)

Detailed results PANGEA VIII

Inspections in postal hubs and other facilities
- 171,304 packages inspected
- 50,852 packages seized

296 individuals arrested for various offences
- Selling medicines without a license
- Operating a clandestine laboratory producing counterfeits
- Operating websites selling illicit medicines
- Illegal sale of medicines
- Supplying unlicensed medicines

23,530,553 million units seized
- Estimated value of 75.3 million USD
- Seized medicines belong to over 20 different therapeutic categories
- Erectile dysfunction (ED) medicines most commonly seized

2,700 websites shut down (domain name or payment facility removed)
- 546 adverts also removed from auction websites and social media platforms

DNA-based Identification Enables Metabolomics-Driven Detection of Botanical Adulteration

- Dr. Charlotte Simmler
- Research Associate Professor
- UIC Center for Natural Product Technologies, Department of Medicinal Chemistry and Pharmacognosy, Chicago, Illinois

DNA barcoding with metabolomics

Authentication methods for licorice botanicals

DNA-based Identification Enables Metabolomics-Driven Detection of Botanical Adulteration

- Authenticity

Identity
- Morphology
- Genome

Part of the plant

Phytochemistry

DNA barcoding

- rbcL
- matK
- trnH-psbA
- ITS2

Metabolomic analysis

DNA verified samples of single species

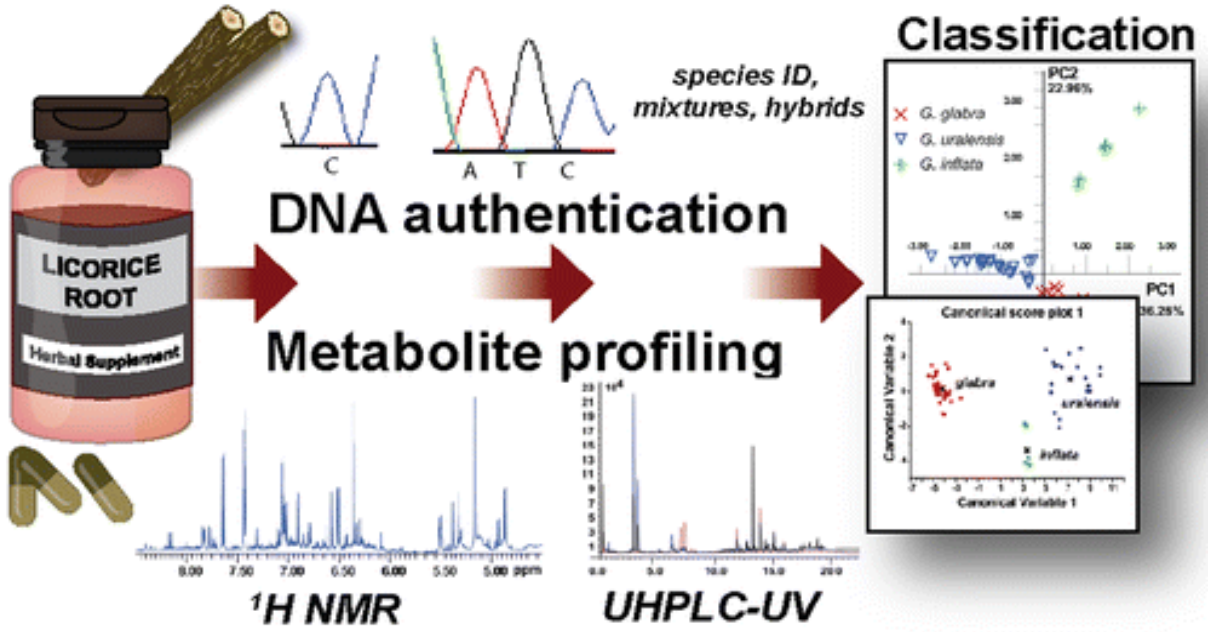
DNA-based Identification Enables Metabolomics-Driven Detection of Botanical Adulteration

Authentication methods for licorice botanicals

- Licorice can be obtained from mainly 3 Glycyrrhiza species
  - G. glabra, G. uralensis, and G. inflata
- Major results

Samples	51
Single species	37
Hybrids	6
Mixtures	8

11 samples were mislabeled



Confirming Species Identity of Herbal Dietary Supplements, An Example from Devil's Claw

- Damon Little, Ph. D
- Associate Curator
- The New York Botanical Garden, Bronx, New York
- Developing DNA barcoding assays with the aim of making barcoding techniques more widely accessible to non–specialists.

Herbal supplement preparations

Objective of DNA identification

An example from Devil's Claw

Confirming Species Identity of Herbal Dietary Supplements, An Example from Devil's Claw

Objective of DNA identification

	"Universal"	"targeted"
Advanced knowledge of the sample required	no	Yes
Accurate species identifications produced	rarely	Usually
Able to detect the unexpected	Usually	Rarely
Demonstrate absence of a species	Very difficult	Possible
Produces meaningful quantitative data	no	Possibly
replicable	Usually	Usually

Confirming Species Identity of Herbal Dietary Supplements, An Example from Devil's Claw

Objective of DNA identification

- Are adequate reference sequences available
- Enough individuals to estimate (within or among species)
- Are all of the close relatives sampled (look-alike or common substitutes)
- Can the target species be told apart using the chosen DNA segments

Confirming Species Identity of Herbal Dietary Supplements, An Example from Devil's Claw

An example from Devil's Claw

- 獅子王球屬(*Harpagophytum* spp.)
- Only 2 species : *Harpagophytum procumbens* and *H. zeyheri*
- Recent studies demonstrate that *H. procumbens* and *H. zeyheri* are chemically distinct and should not be treated as equal.
- Further, the sale of *H. zeyheri* as an herbal supplement is not legal in the United States.

Confirming Species Identity of Herbal Dietary Supplements, An Example from Devil's Claw

An example from Devil's Claw

- Chloroplast psbA-trnH gene
- Major results

PCR amplification succeeded	19 samples
Only *H. zeyheri*	16 (84%)
Both *H. zeyheri* and *H. procumbens*	3 (16%)
Only *H. procumbens*	0

- This novel mini–barcode as a standard method of quality control in the manufacture of Devil's Claw supplements

Meat Speciation

- Geoffrey COTTENET
- A scientist at Nestlé Research Center in Lausanne, Switzerland
- The development and validation of molecular methods
  - for the detection and quantification of GMO
  - for authenticity (meat, fish and plant species identification)

Food Fraud

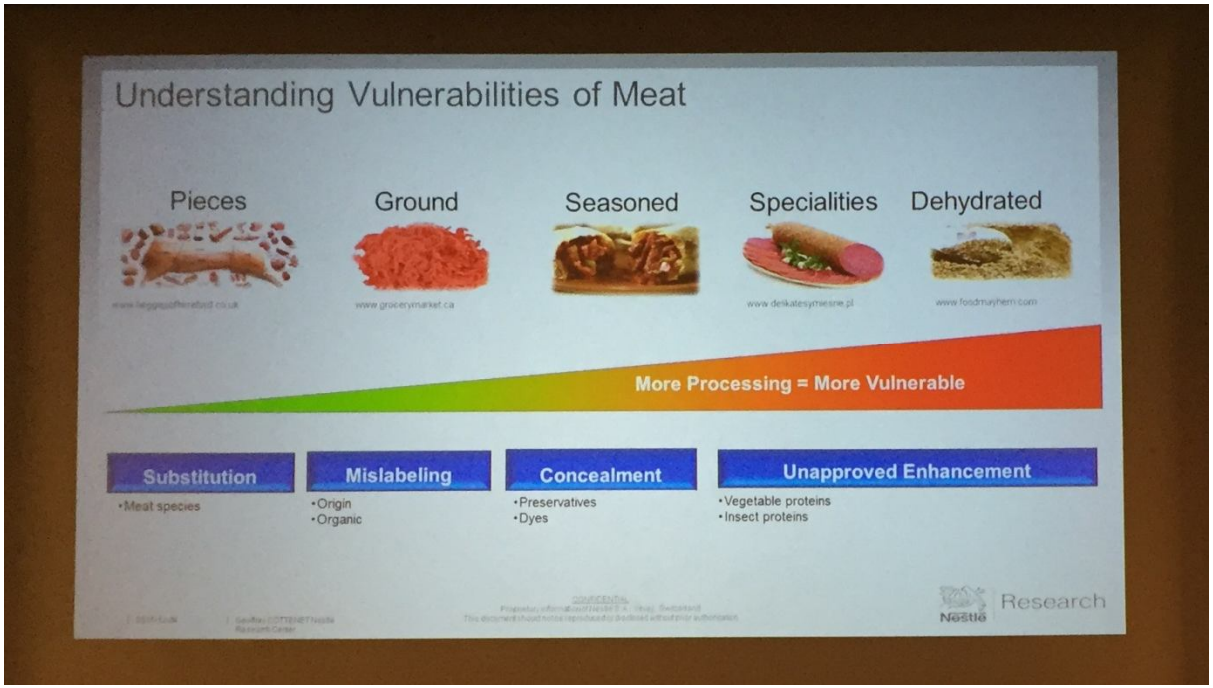
Limitation of PCR methods

Meat LCD Array

Meat Speciation

Food Fraud

- 2013 Europe: beef adulterated with horse meat
- DNA-based methods and especially PCR techniques



Meat Speciation

Limitation of PCR methods

- Limited to maximum 5-6 meat species (beef, pork, horse, chicken and turkey)
- The latest Chinese cases of meat substitution where fox and rat meat were detected instead of donkey and lamb

Meat LCD Array

- A simultaneous screening of 32 meat species with a detection of meat mixtures ≤ 1% (m/m)
- Easy to use, fast, and cost efficient

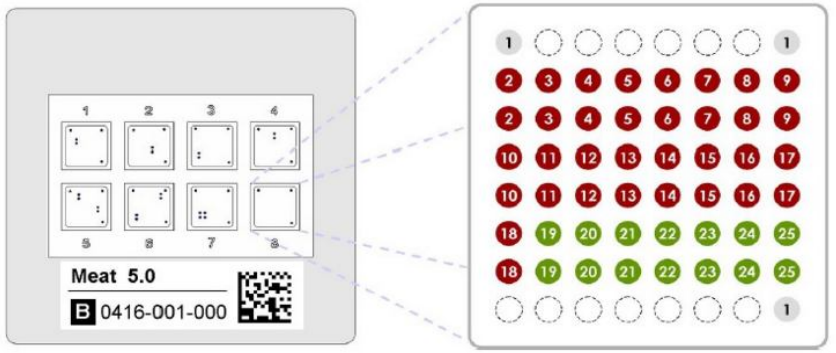
Meat Speciation

Meat LCD Array

Chipron Meat 5.0 LCD K

One experiment to know 24 animal species

–Cover 24 species in one experiment,
–Perfect experiment for First Round screen
–Find unexpected additions of animal material. Traditional method cannot offer!



No.	Name	*Species*	No.	Name	*Species*
01	Hyb-Ctrl	Hybridization Control			
02	Cattle	*Bos taurus, Bos bison*	14	Red Deer *	*Cervus elaphus*
03	Sheep	*Ovis aries*	15	Fallow Deer	*Dama dama*
04	Equine	*Equus caballus, E. asinus*	16	Springbok	*Antidorcas marsupialis*
05	Goat	*Capra hircus*	17	Canine / Dog	*Canis sp.*
06	Camels	*Camelus sp.*	18	Cat	*Felis silvestris*
07	Buffalo	*Bubalus bubalis*	19	Chicken	*Gallus gallus*
08	Pork	*Sus scrofa*	20	Turkey	*Meleagris gallopavo*
09	Kangaroo	*Macropus rufus / giganteus*	21	Goose	*Anser sp.*
10	Hare	*Lepus europaeus*	22	Ostrich	*Struthio camelus*
11	Rabbit	*Oryctolagus cuniculus*	23	Mallard Duck	*Anas platyrhyncos*
12	Reindeer *	*Rangifer tarandus*	24	Muscovy Duck	*Cairina moschata*
13	Roe Deer	*Capreolus capreolus*	25	Pheasant	*Phasianus sp.*

* Weak cross reactivity of the capture probe for Reindeer with pure Red Deer and vice versa can occur at high target

Fish Speciation

- Dr. Jonathan Deeds
- Research Biologist
- U.S. Food and Drug Administration College Park, Maryland
- As a research coordinator and subject matter expert in the areas of seafood safety and labeling

Seafood labeling

Project Fish SCALE (Seafood Compliance and Labeling Enforcement)

Fish Speciation

Seafood labeling

- Over 90% of the seafood in the U. S. marketplace is imported
- Accurate labeling is a critical first step
  - Potential risks (natural toxins, allergens, etc.)
- Guidance for Industry: FDA's Guide to Acceptable Market

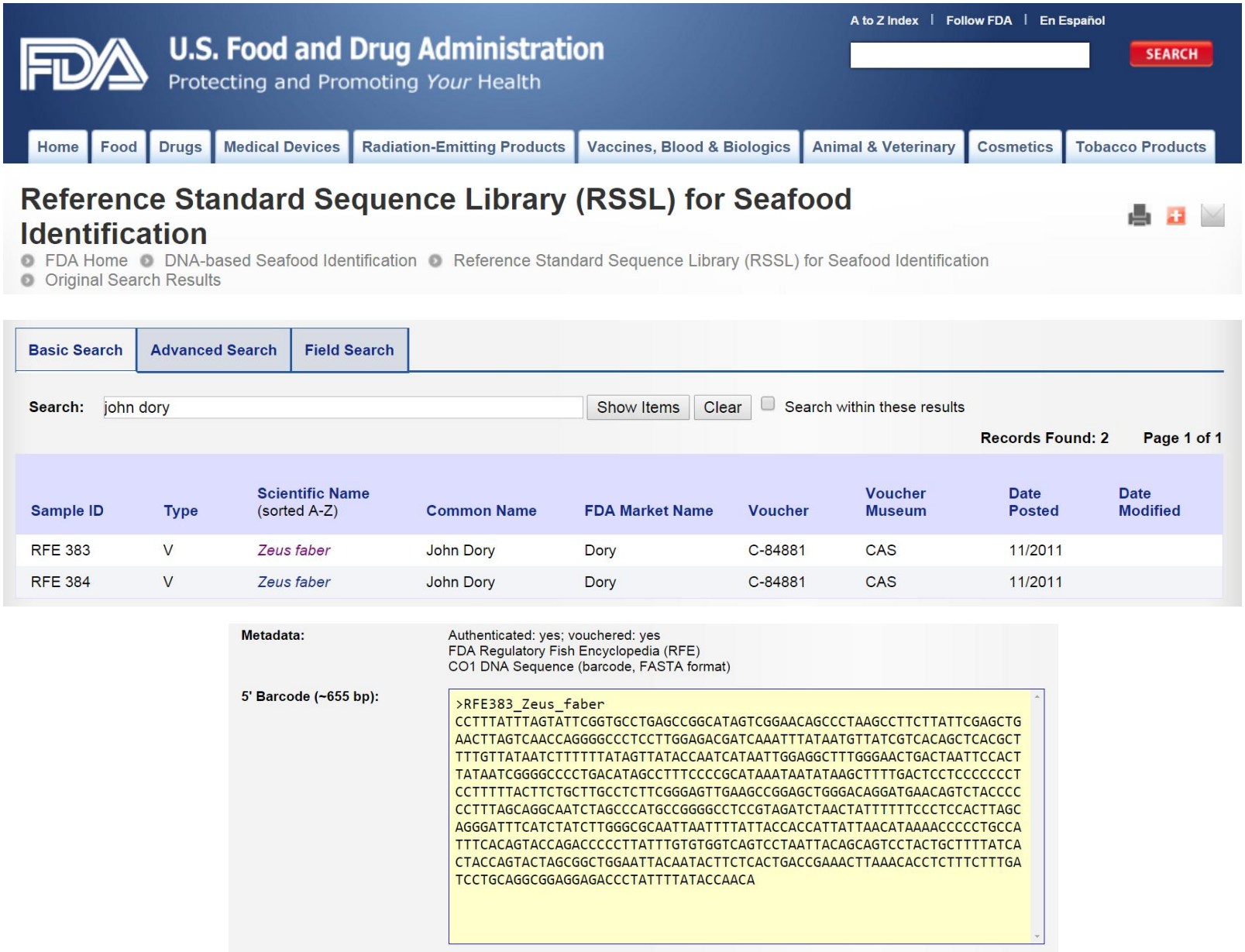
Column A	Column B
Various Snappers (*Lutjanus* spp.)	**Red Snapper** (*Lutjanus campechanus*)
Rockfish (*Sebastes* spp.)	**Red Snapper** (*Lutjanus campechanus*)

Fish Speciation

Project Fish SCALE

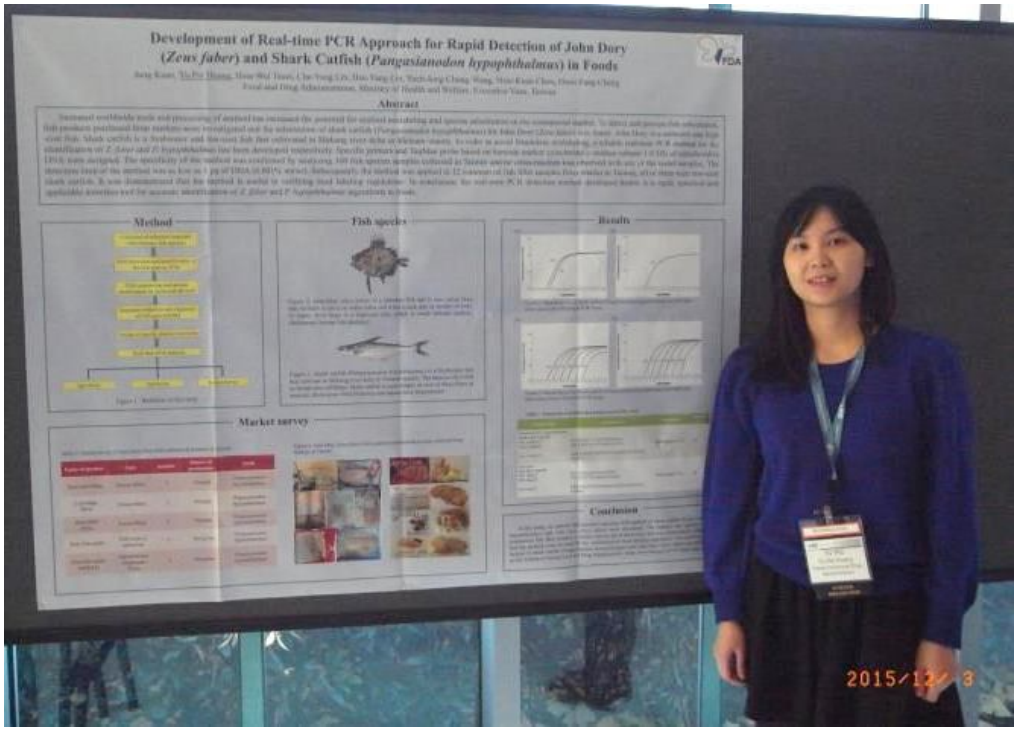
- In collaboration with the Office of Food Safety (OFC), Office of Compliance (OC), and Office of Regulatory Affairs (ORA)
- Seafood labeling and species identification
- At the heart of this project
  - The updating of FDA's species identification capabilities to modern forensic techniques using DNA sequencing

Fish Speciation



壁報論文展示

Development of Real-time PCR Approach for Rapid Detection of John Dory (*Zeus faber*) and Shark Catfish (*Pangasianodon hypophthalmus*) in Foods



壁報論文展示

The Analysis Report of Status for Chinese Herbs Misuses in 2014~2015

壁報論文展示

- **DNA barcoding for identification of species in mushrooms: A component of product certification**
- **The Utility of DNA Authentication for the Metabolomic Classification and Quality Control Of Licorice Botanicals**
- **Development of a Non-targeted, Raman Detection Method for Authentication of Milk Powders**

Thanks for

your attention