TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory

Drug Testing and Residue Avoidance in Show Animals

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Section Head
Analytical Chemistry
(Drug Testing & Toxicology)

Overview of the DTL

- Established in 1989
  - Legislative direction to provide drug testing for pari-mutuel horse racing
  - ~2,800 blood and urine samples annually

- 1992
  - Legislative direction to provide drug testing for pari-mutuel greyhound racing
  - ~400 urine samples annually

- Between 1989 - 1992
  - Began providing drug testing for livestock shows and county fairs
  - ~3,200 samples annually

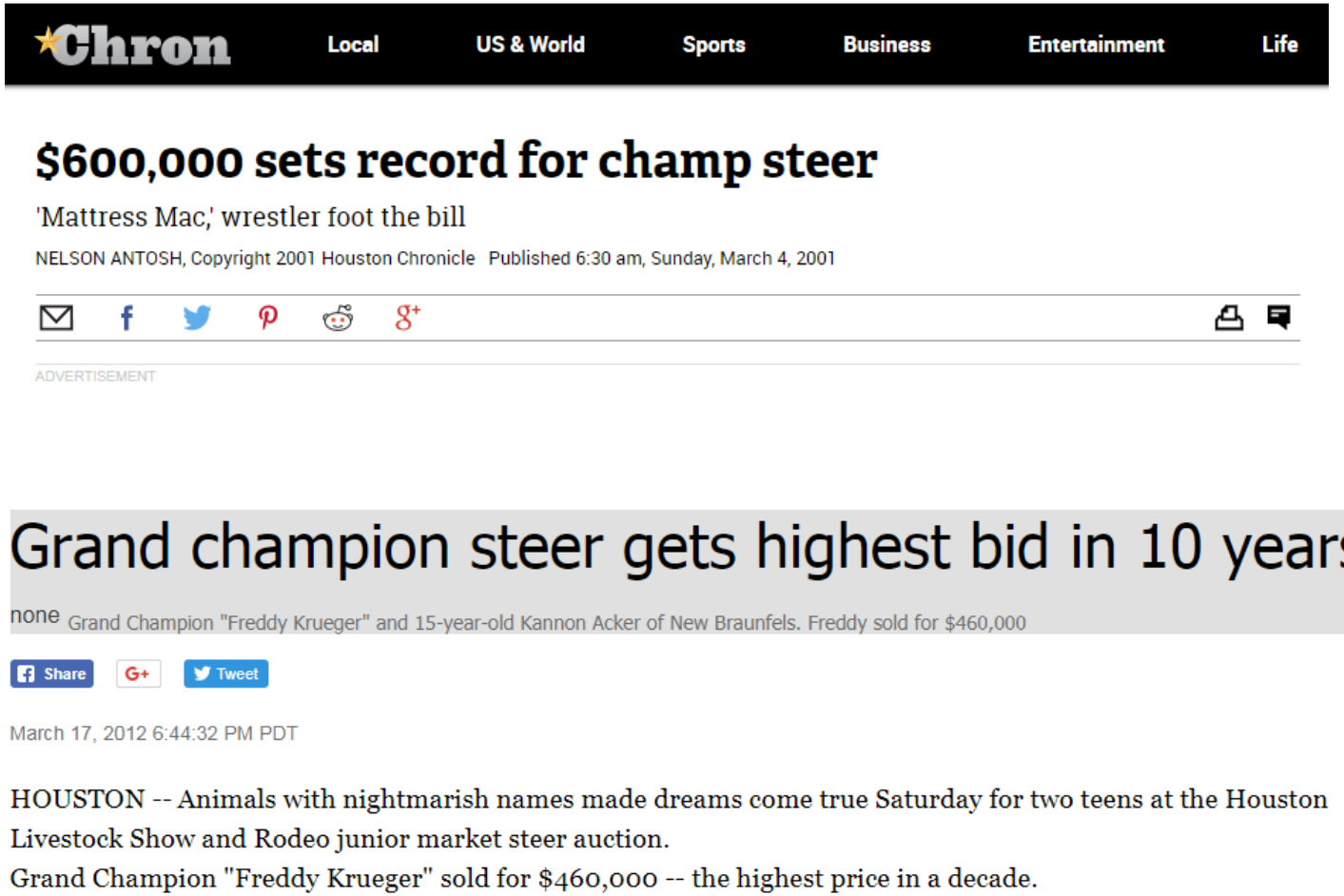
Other Services (tvmdl.tamu.edu)

- Equine Pre-Purchase Drug Screen
  - NSAIDs
  - Long-Acting Tranquilizers
- Beta-Agonists
- Corticosteroids
- Anabolic Steroids
- Split Sample Testing
- Drugs of Abuse
- Therapeutic Drug Monitoring
  - Keppra® (Levetiracetam)
  - Zonegran® (Zonisamide)
  - Itraconazole
- Equine Hair Drug Screen
  - Ractopamine, Clenbuterol, Zilpaterol, Albuterol
- General Drug Screen

Drug Testing in Show Animals

Why Testing is Important

- Foster fair competition
- Animal welfare
- Food safety
- Increase premiums
- Increase entries



Chron Local US & World Sports Business Entertainment Life

$600,000 sets record for champ steer

'Mattress Mac,' wrestler foot the bill

NELSON ANTOSH, Copyright 2001 Houston Chronicle Published 6:30 am, Sunday, March 4, 2001

ADVERTISEMENT

Grand champion steer gets highest bid in 10 years

none Grand Champion "Freddy Krueger" and 15-year-old Kannon Acker of New Braunfels. Freddy sold for $460,000

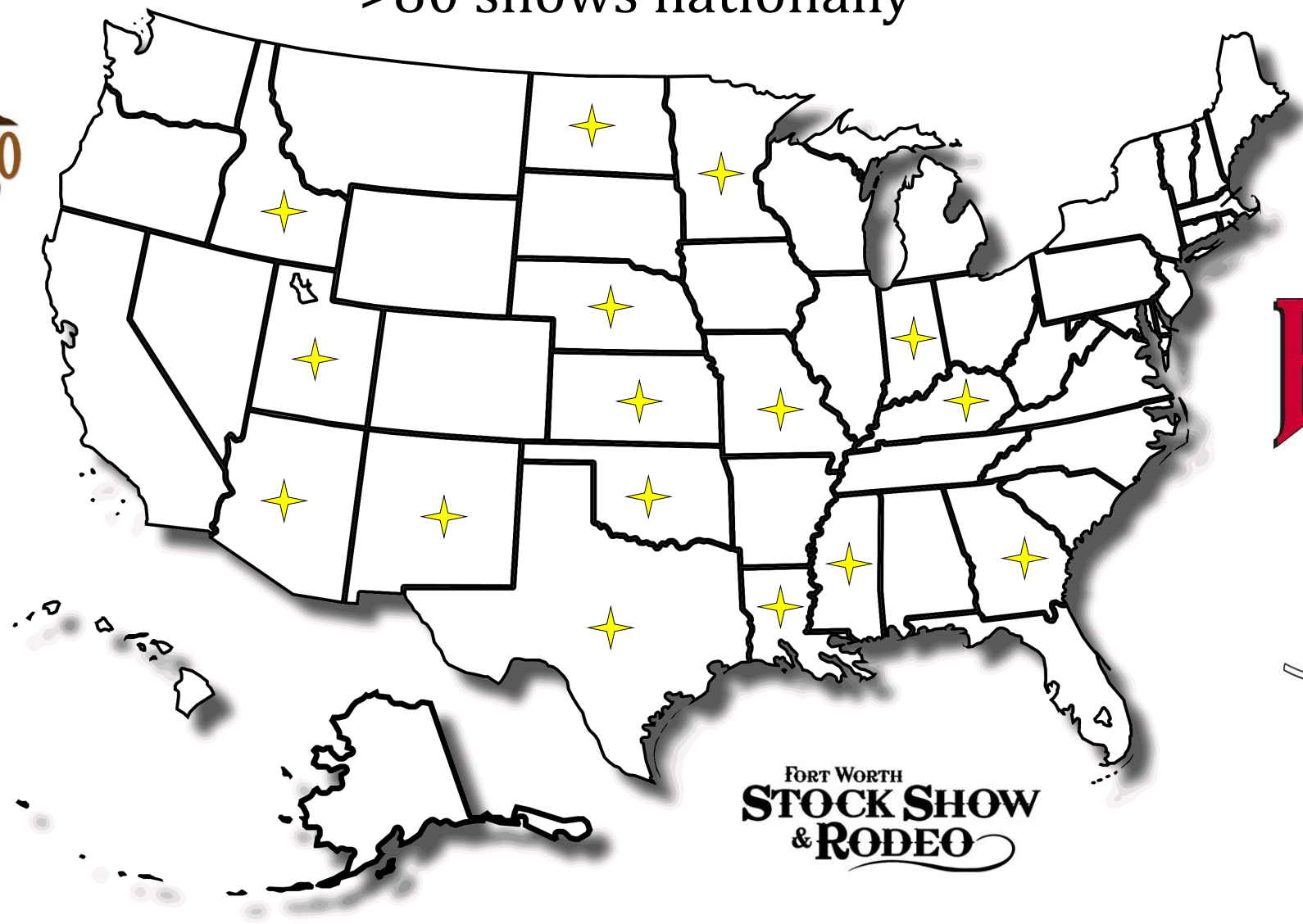
Share G+ Tweet

March 17, 2012 6:44:32 PM PDT

HOUSTON -- Animals with nightmarish names made dreams come true Saturday for two teens at the Houston Livestock Show and Rodeo junior market steer auction.

Grand Champion "Freddy Krueger" sold for $460,000 -- the highest price in a decade.

>80 shows nationally



San Angelo Stock Show & Rodeo

Rodeo Austin

San Antonio Stock Show & Rodeo

Heart O' Texas Fair & Rodeo presented by H-E-B

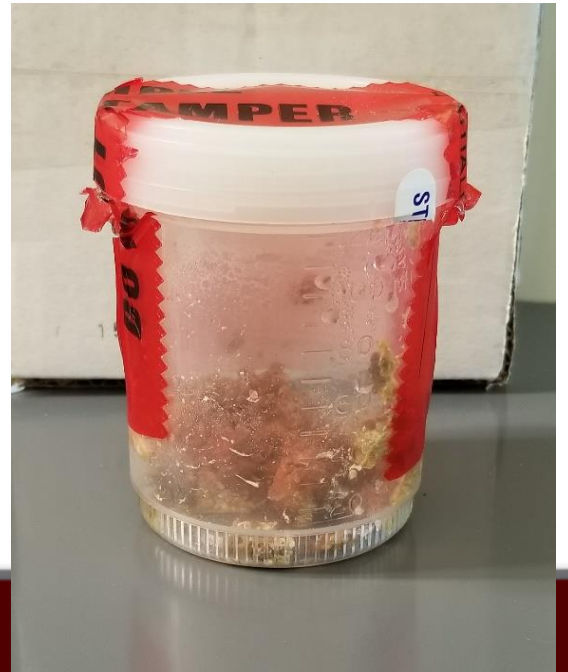
State Fair of Texas

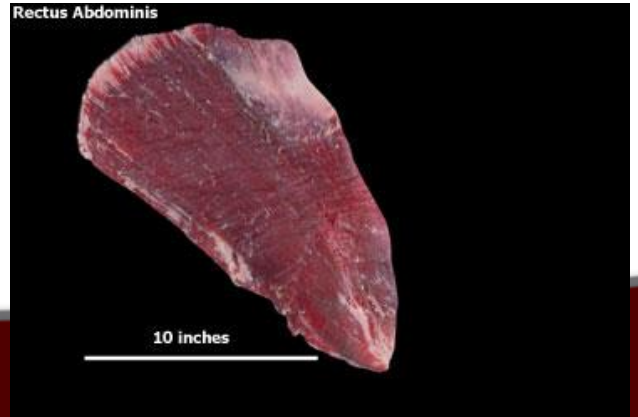
Houston Livestock Show and Rodeo

Fort Worth Stock Show & Rodeo

TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory


PSA-RMC
Potassium-RMC




Rectus Abdominis
10 inches

What Do We Test For??

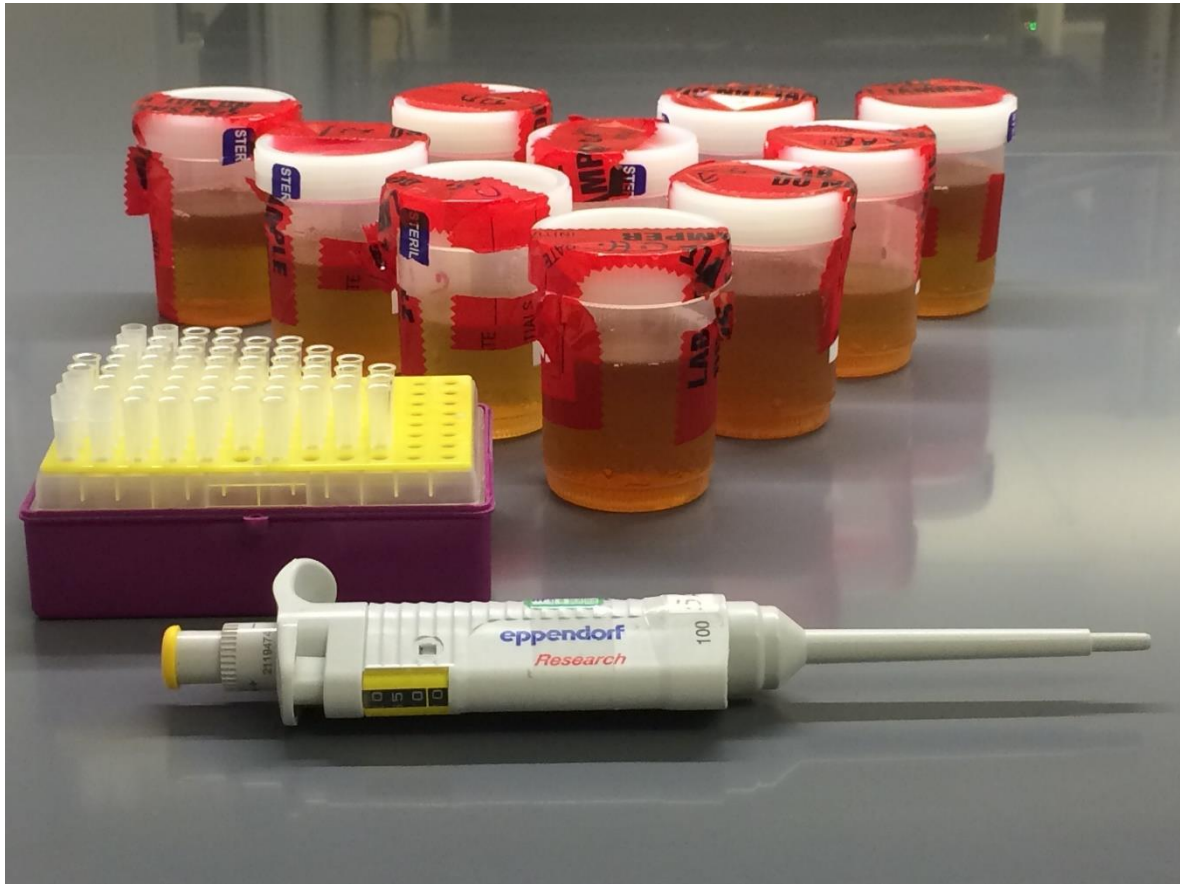
- NSAIDs
- Growth Promoters/Repartitioning Agents
- Steroids
- Analgesics
- Stimulants
- Muscle Relaxers
- Illicit Drugs
- Sedatives/Tranquilizers

What We Don't Test For

- Antimicrobial Drugs
- Avermectins
- Ionophores
- Antifungal Drugs
- Herbal and All-Natural Products

**The main ingredient may not test but other ingredients might

Drug Testing Process



Chain of Custody

vs.

College Station Lab
483 Agronomy Rd.
College Station, TX 77840
(979) 845-3414

Stock Show Submission Form

Document: 35271
Revision: 2

Accession #

Show Name _____

Account # _____

Submitter Name _____

Phone ( ) _____

Email _____

*Please attach a list of animal ID numbers and animal species (**Do Not Include Exhibitor Names**)*

*There will be an additional fee ($25 per sample) to confirm and report Ractopamine in steers (Optaflexx™) and market barrows (Paylean®): ☐ **Confirm** ☐ **Do Not Confirm**

(Note: this drug will be confirmed at no additional charge if detected in unapproved species)

I certify that these samples were collected, packaged, and shipped in a manner as to maintain integrity, chain of custody, and avoid opportunities for contamination:

Submitter: _____ (Print) _____ (Sign) _____ (Date)

All samples submitted to TVMDL for testing become the property of the agency and may be tested as part of state/federal surveillance programs, utilized for research purposes and/or development of new assays. TVMDL is unable to return samples to the client unless prior arrangements are made and approved by the agency Director or designee.

Lab Use Only

Date Received: _____ Sample Condition: ☐ Frozen ☐ Chilled ☐ Ambient

Were Sample Seals Intact? ☐ Yes ☐ No

Describe any additional details: _____

Lab Personnel: _____ (Print) _____ (Sign) _____ (Date)

Return completed form to the email address above

TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory	DTL Sample Information Form #____of____			
	Classification	ID	Revision	Effective Date
	S-DTL-F	9656	16	6/6/2017 1:26:29 PM

SUBMIT THIS FORM FOR EACH SAMPLE, INCLUDING MULTIPLE SAMPLES WITH THE SAME ACCESSION NUMBER

SAMPLE INFO

Accession #: _____ **Race Date (N/A):** _____ **Date Received:** _____

Sample #: _____ **Date Due:** _____ **Submitted By:** _____ **Date:** _____

Sample Matrix: ☐ *Serum* ☐ *Urine* ☐ *Liver* ☐ *Other:* _____

Test Requested: ☐ *NSAIDs* ☐ *Pre-Purchase* ☐ *Drugs of Abuse** ☐ *General Drug Screen* ☐ *Livestock Show* ☐ *Tranqs**

☐ *Racing (EQ K9)* ☐ *Contraband* ☐ *Vet Work* ☐ *Graded Stakes* ☐ *Other:* _____

☐ *Split Sample (Suspect for:* _____ *Concentration:* _____)

SCREENING ANALYSIS

Extraction SOP: ☐ #9703 Simple (Neutral Basic Acidic) ☐ #9700 Tranqs ☐ #9692 AU ☐ #9694 BU ☐ #9706 NU

☐ #9697 EH ☐ #10142 Drug Screen Extraction ☐ #9697 EH + #9709 SPE ☐ Other: _____

Spike (HRM) Lot #/volume: _____

Internal Standard Lot#/ volume: _____

Submitted to Instrumental Analysis by: Initials: _____ Date: _____

Residue re-suspended in (solvent) _____ (Vol.) _____ Initials: _____ Date: _____

Screening Result(s): ☐Negative ☐Suspect For: _____

☐ Spike Detected (if applicable) Screened By: _____ Date: _____ Verified By: _____ Date: _____

Instrument: ☐ QQQ-1 ☐ QQQ-2 ☐ OrbiTrap ☐ Endura ☐ GC/MS

File Location: _____

CONFIRMATION ANALYSIS

Extraction SOP: ☐ #9703 Simple (Neutral Basic Acidic) ☐ #9700 Tranqs ☐ #9692 AU ☐ #9694 BU ☐ #9706 NU

☐#9697 EH ☐ #9709 SPE ☐ #10142 Drug Screen Extraction ☐ #9697 EH + #9709 SPE

☐Other: _____ pH (if applicable) _____

Spike Information: Lot #/volume: _____

Internal Standard Information: _____

Submitted to Instrumental Analysis By: Initials: _____ Date: _____

Residue re-suspended in (solvent) _____ (Vol.) _____ Initials: _____ Date: _____

Confirmation Result(s): ☐Negative ☐Confirmed: _____

Conc. (if applicable): _____ ☐ Spike Detected

Confirmed By: _____ Date: _____ Verified By: _____ Date: _____

Instrument: ☐ QQQ-1 ☐ QQQ-2 ☐OrbiTrap ☐Endura ☐GC/MS Conc. Of HRM (if applicable):_____

File Location: _____

Data Included:

- ☐ Calibration Curve
- ☐ Sample Sequence (Initial)
- ☐ Sample Sequence (Confirmation)
- ☐ MS Results Spreadsheet (#9720)
- ☐ Spike Controls (#9680)

Chromatograms (Including ion ratios and ISTD where applicable):

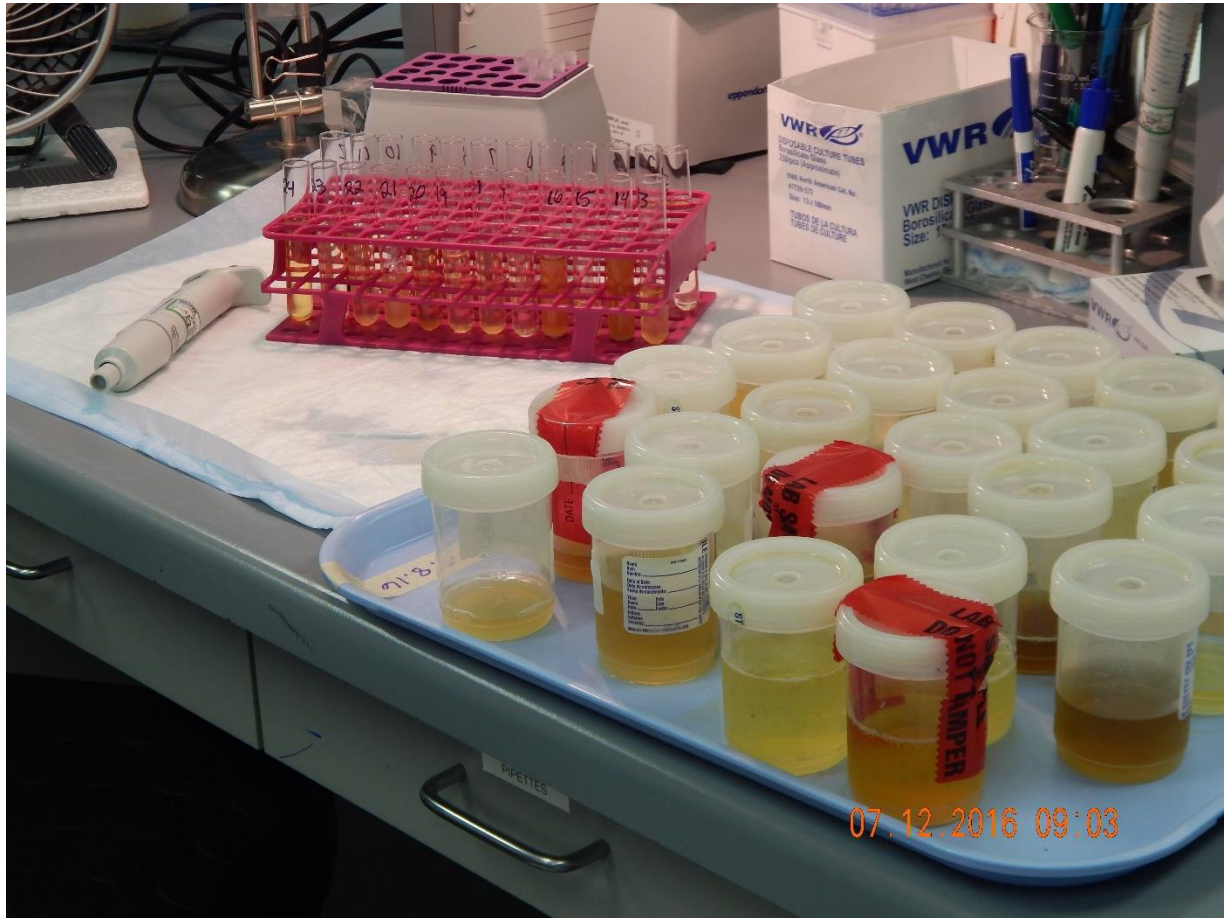
- ☐ Sample (Initial)
- ☐ Sample (Confirmation)
- ☐ Reference STD
- ☐ NCU/NEG Serum
- ☐ HRM/Spike/QC
- ☐ Solvent Blank (preceding sample injection)
- ☐ All Calibrators

*The Drugs of Abuse Screen Includes: Amphetamine, Barbiturates, Benzodiazepines, Cocaine, Benzoylecgonine, Ketamine, Lidocaine, Hydroxylidocaine, Methamphetamine, Methylphenidate, Opiates, Phencyclidine, Promazines, THC/metabolites

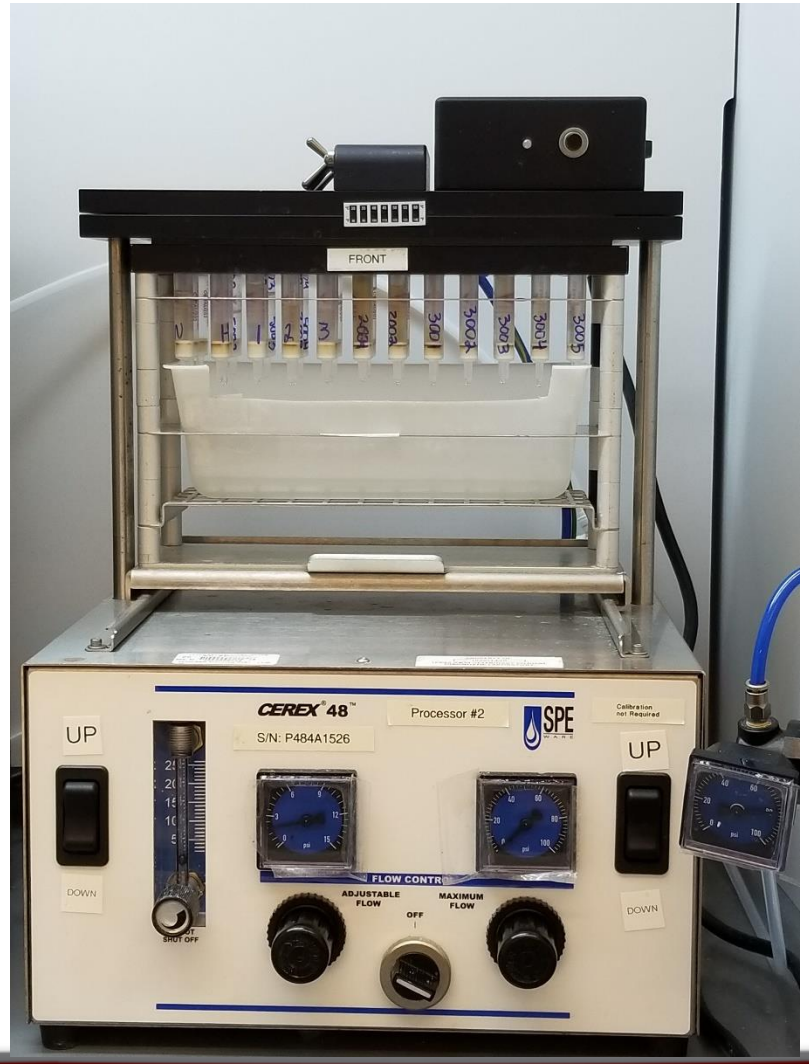
*The Tranquilizer Screen Includes: Fluoxetine, Fluphenazine, Reserpine, Acepromazine, Guanabenz, Romifidine

ECCN:

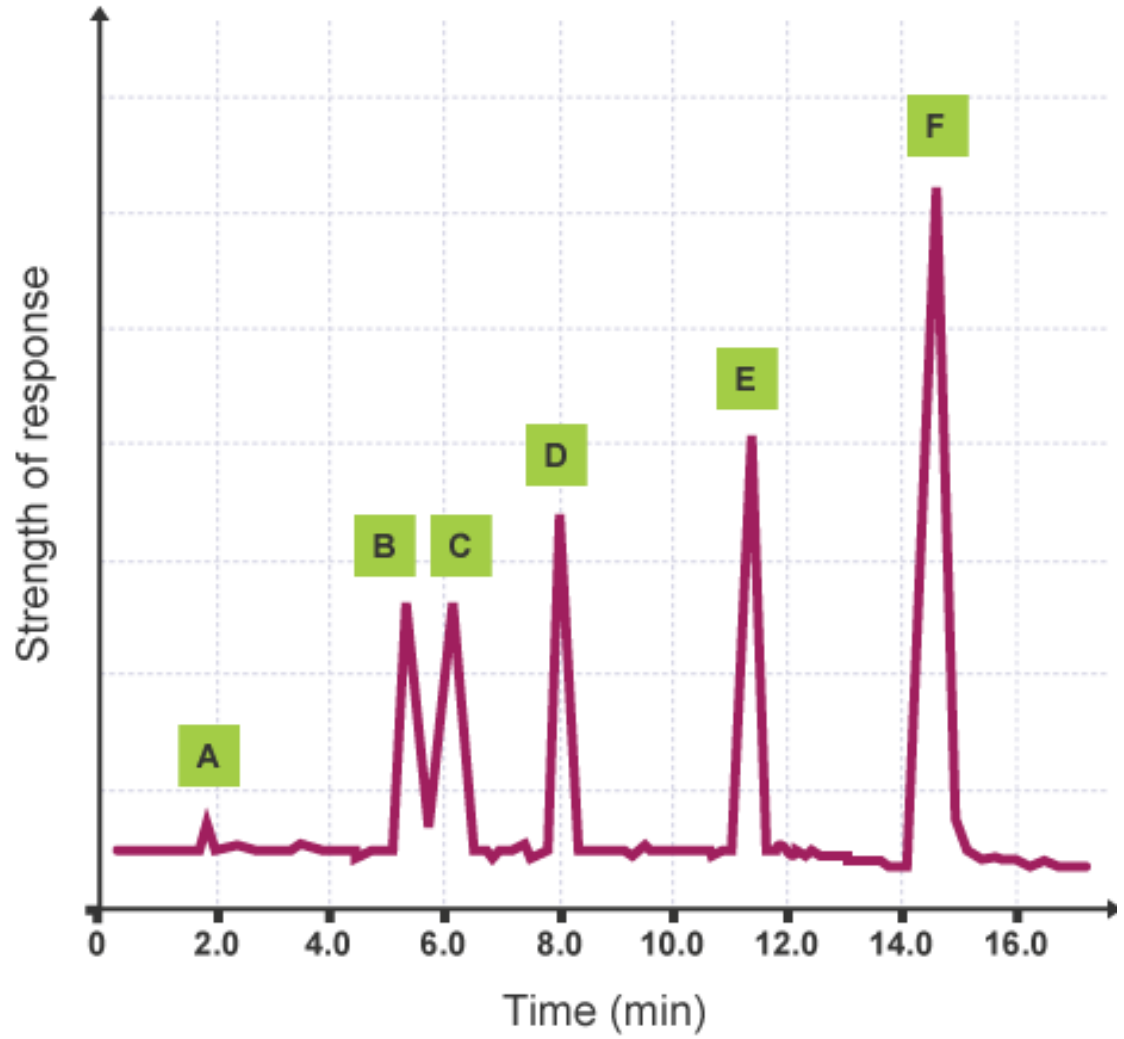
Sample Preparation

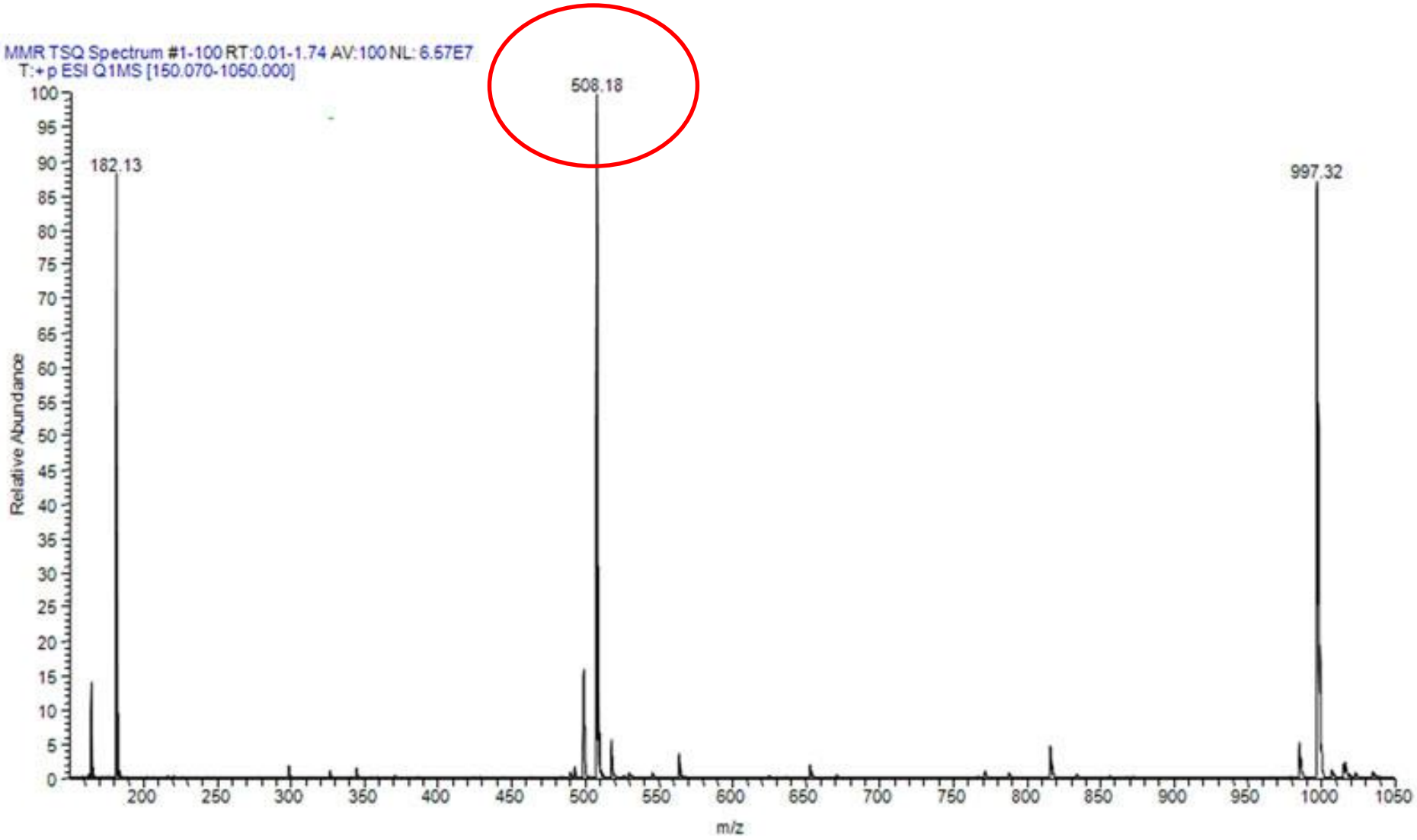


Sample Extraction

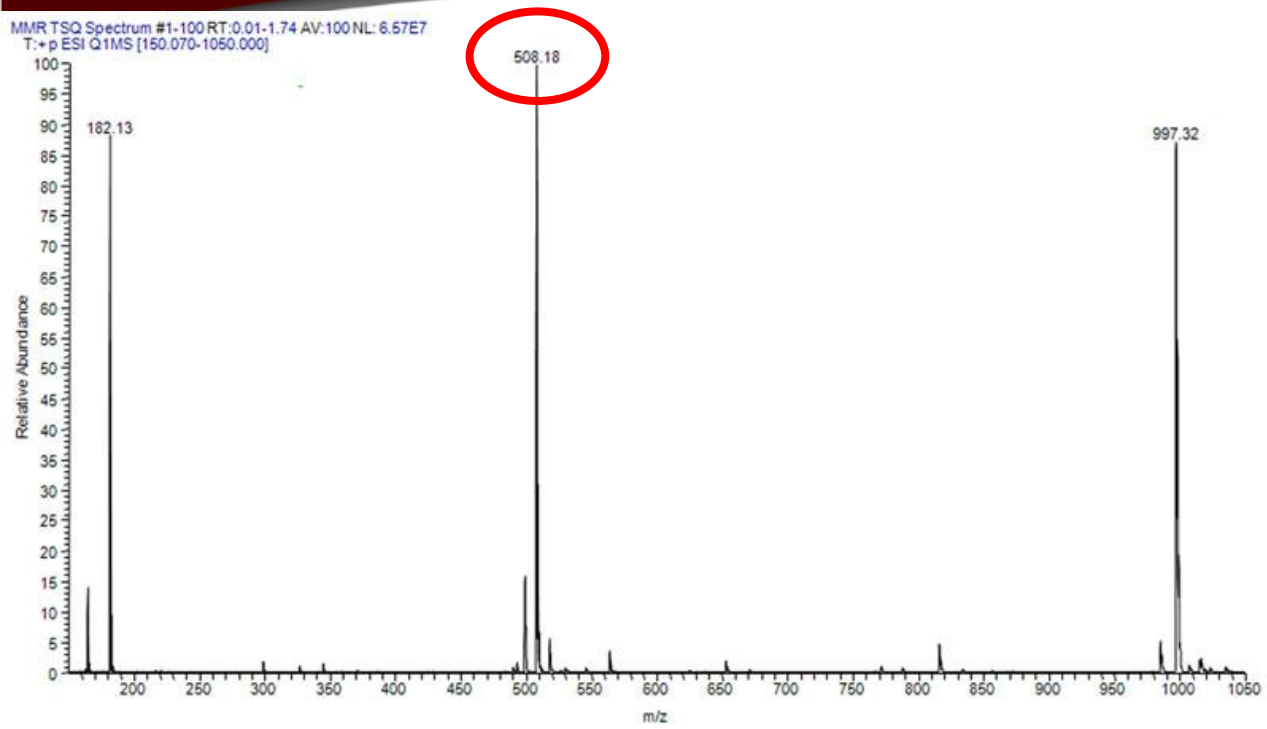


Screening


Strength of response
A
B
C
D
E
F
0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Time (min)


MMR TSQ Spectrum #1-100 RT:0.01-1.74 AV:100 NL: 6.57E7
T:+p ESI Q1MS [150.070-1050.000]
508.18
182.13
997.32
Relative Abundance
m/z

Confirmation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182.13
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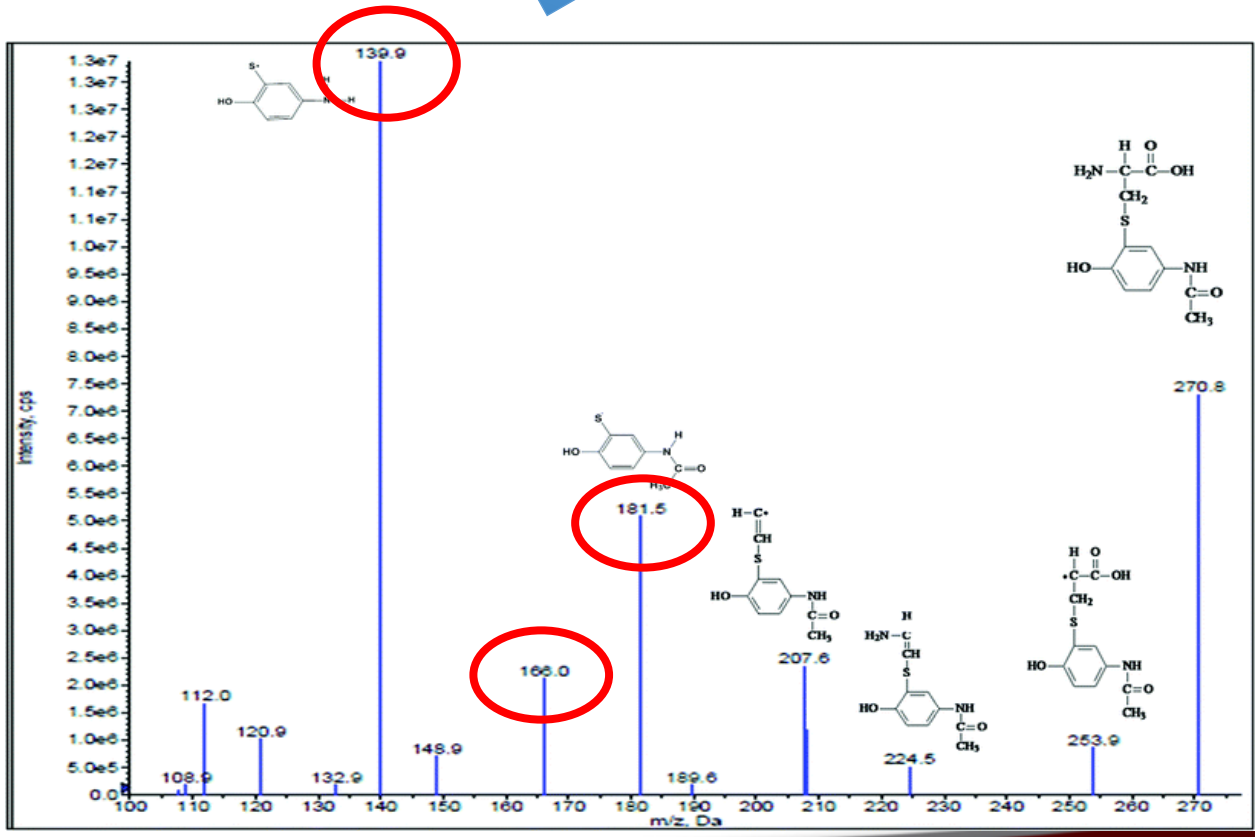

139.9
181.5
166.0
270.8
207.6
112.0
120.9
148.9
132.9
108.9
189.6
224.5
253.9
Intensity, cps
m/z, Da

Reporting Results

- All data is reviewed by at least two individuals
- Results are reported directly to the show
- Qualitative vs. Quantitative
  - Interpretation – incidental vs. intentional
- Sample retention:
  - Negative samples retained refrigerated for ~ 2weeks after reporting
  - Positive samples retained frozen for 1 year
- Records are retained for 2 years

Advancements in Technology

Why??

- Regulatory Testing for the Horse Racing Industry
  - Required to achieve sensitivity to detect therapeutic drugs
    - i.e. Dexamethasone at 5 pg/mL in blood
  - Required to achieve accreditation
    - American Association for Laboratory Accreditation (A2LA) – ISO 17025
    - Racing Medication & Testing Consortium (RMTC)

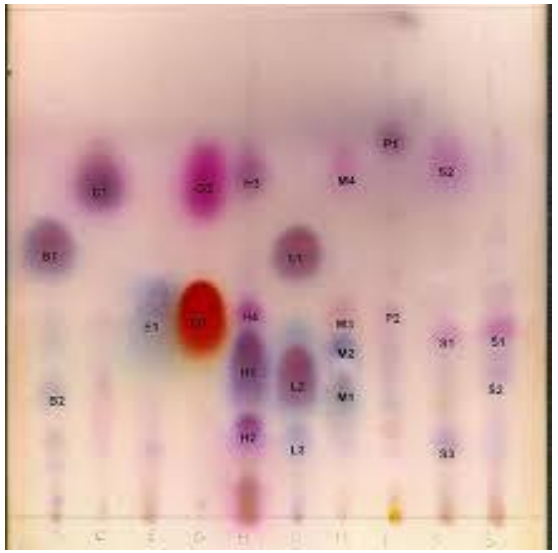
The American Association for Laboratory Accreditation
"World Class Accreditation"

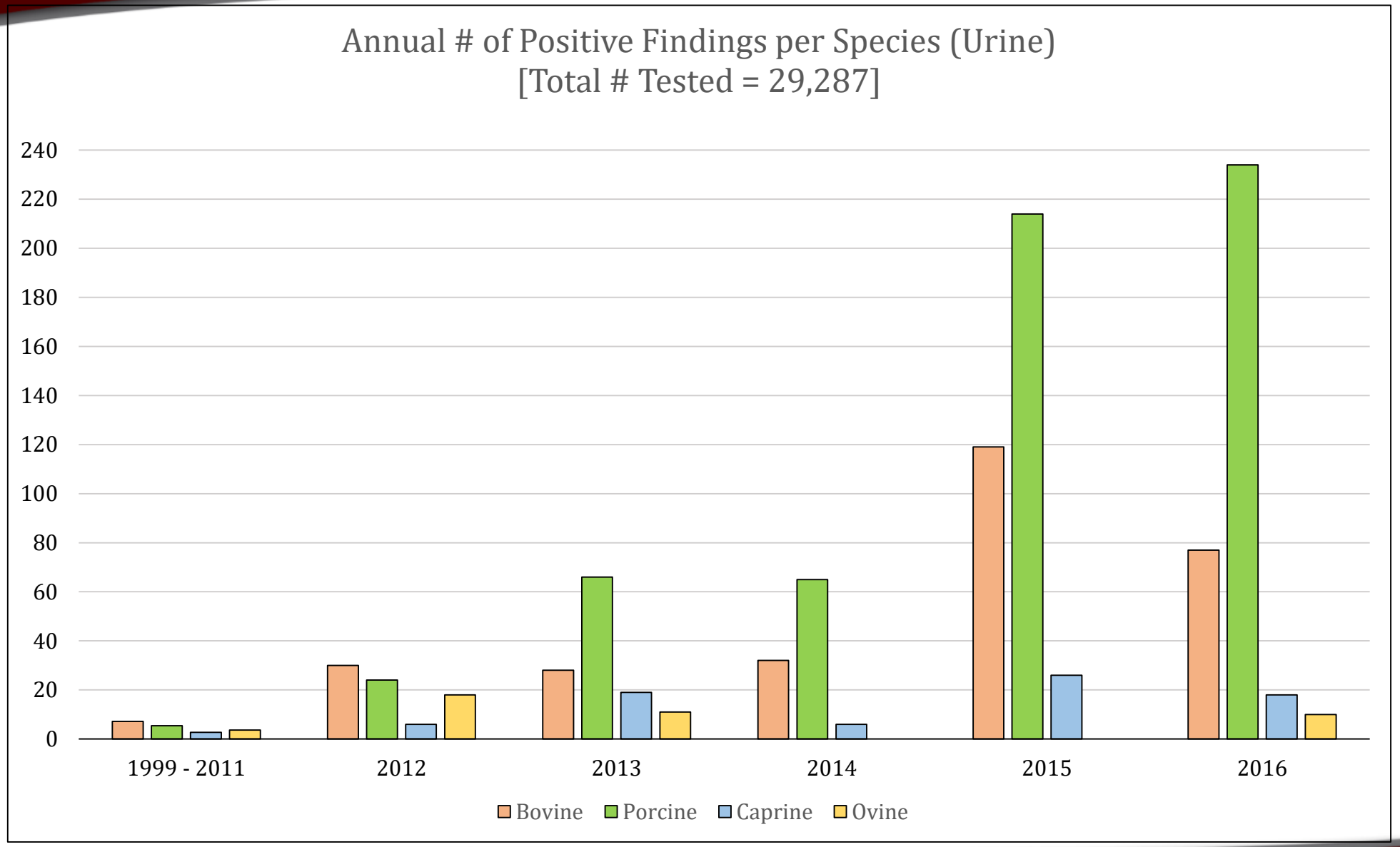
RMTC Racing Medication & Testing Consortium

Applied to Livestock Show Testing

- Improved sensitivity
- Increased sample throughput
- Reduction in false positives at screening
- Elimination of “masking agents”
- Ability to add/remove drugs quickly
- Increased automation
- Access to drug libraries

- Screening “pre-2015”:
  - TLC
  - ELISA
- Screening 2015:
  - LC-MS



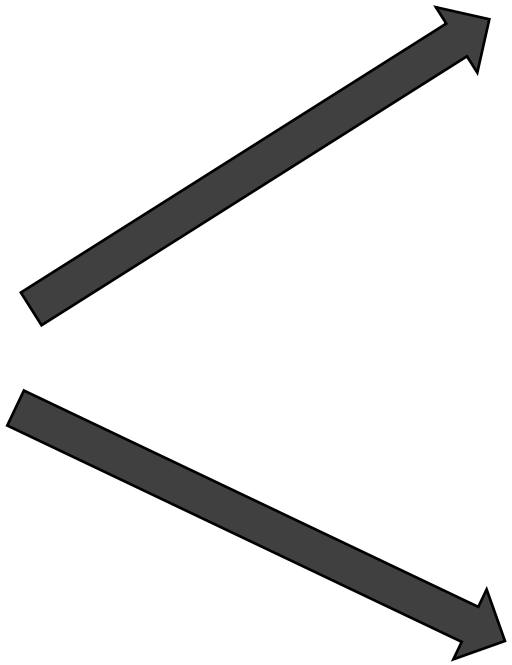

Annual # of Positive Findings per Species (Urine)
[Total # Tested = 29,287]
240
220
200
180
160
140
120
100
80
60
40
20
0
1999 - 2011
2012
2013
2014
2015
2016
Bovine
Porcine
Caprine
Ovine

What about therapeutic drugs?

Zero Tolerance

- "Zero" = the limits of detection of the method used for screening
  - TLC ~250 ppb
  - ELISA ~1-10 ppb
  - LC-MS ~0.01 ppb
- Consideration of classification of Zero Tolerance
  - Illicit drugs
  - Drugs used in un-approved species

Zero Tolerance



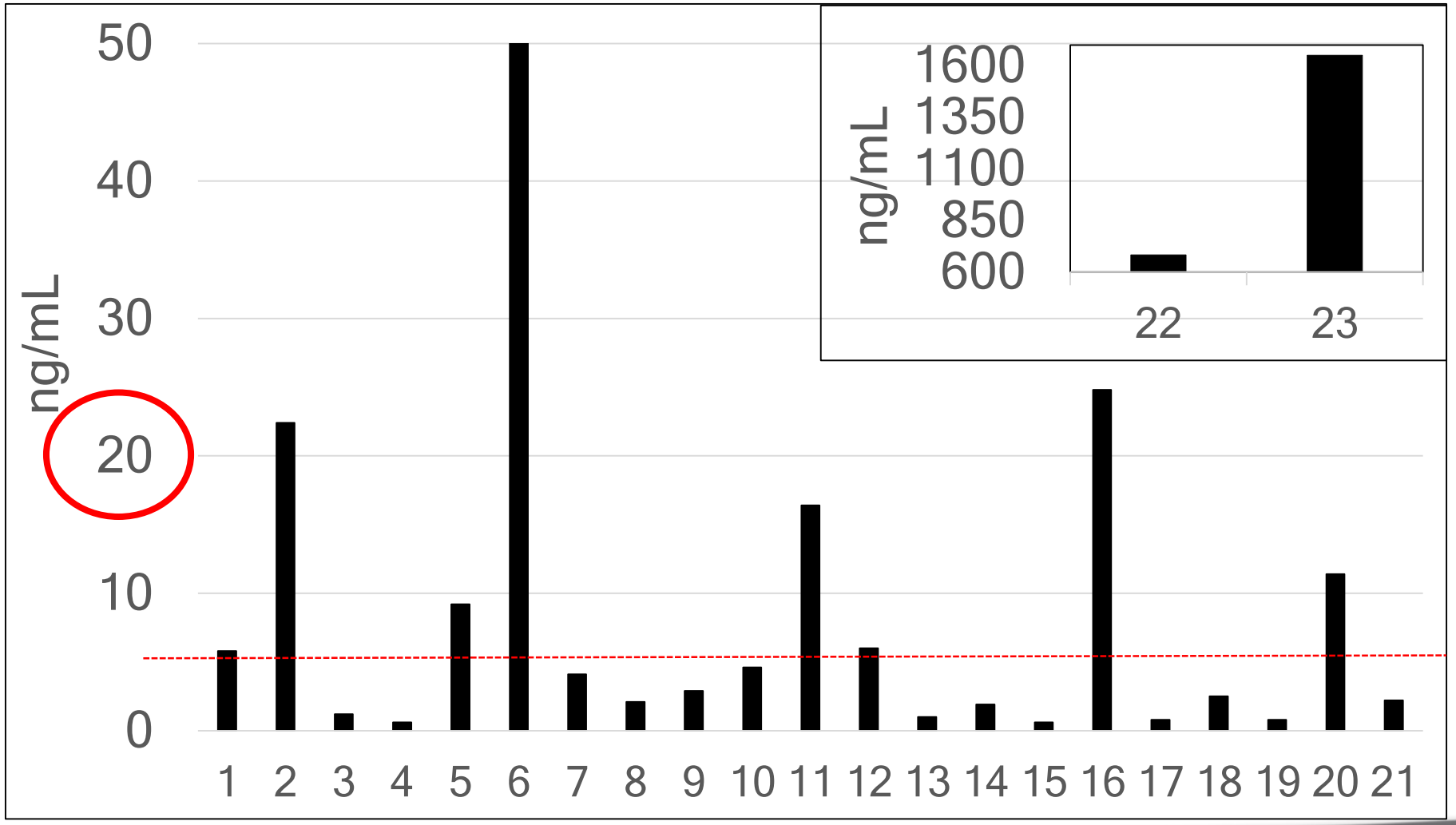
Permissible Drugs

- Therapeutic Drugs
- Threshold levels

Non-Permissible Drugs

- Illicit Drugs
- Off-label use

Flunixin Concentrations in Urine (2016-2017)



Therapeutic Drugs

- Concentration differences between therapeutic and performance-enhancing effect
  - Difficult to measure in a show animal
  - Requires quantitation
- Establish withdrawal guidelines in serum/urine
  - Research related
- Establish screening threshold levels
  - Research related

How to Adjust to Advancements in Testing Capabilities

- Know the show's policies on drug use
- Work with a veterinarian
- Follow the label
- Keep detailed records
- Utilize resources
  - County extension agents
  - Representatives from the show
  - Texas A&M AgriLife Extension
  - TVMDL
  - FARAD (www.farad.org)
  - FDA (https://animaldrugsatfda.fda.gov)

Current Research Efforts and What the Future Holds

Therapeutic Drugs

Background

- Veterinarians need to be able to provide good guidance to their clients with show animals that does not jeopardize their ability to enter shows at the expense of animal health.
- There is a need to be able to defend this approach to the shows, and demonstrate how to approach governance of drug use with useful and evidence-based methods.
- Meat withdrawal times can underestimate drug residues in urine for some drugs.

Study #1

- December 2017
- PK study of Flunixin and Meloxicam in pigs and goats
- Urine, blood and liver samples collected at multiple time points

Study #2

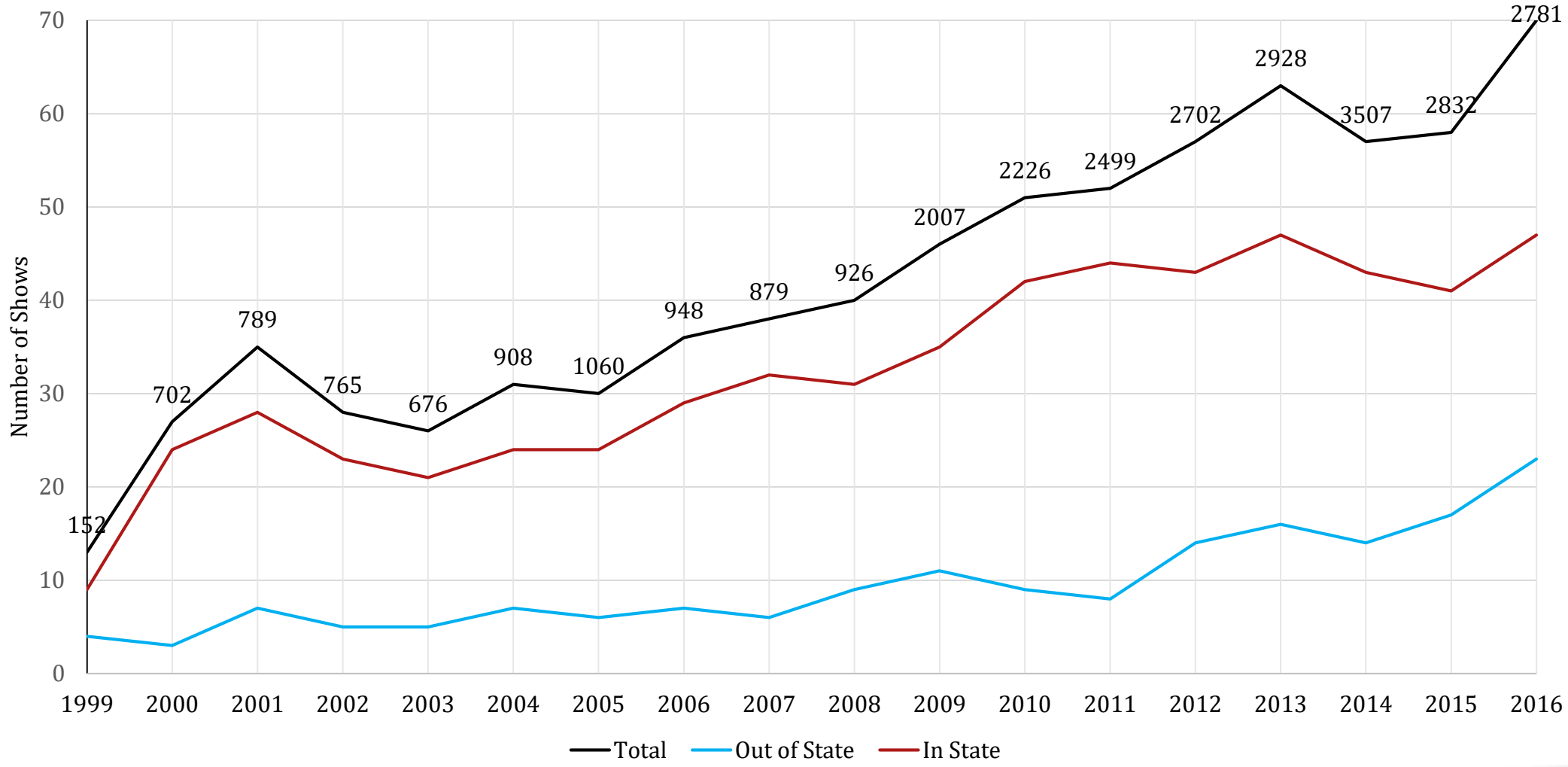
- Early 2018
- PK study of Flunixin and Meloxicam in cattle
- Urine, blood and liver samples collected at multiple time points

Goals and Objectives

- Determine when urine residues of flunixin and meloxicam are below detectable levels
- Determine a conservative urine withdrawal time for these two drugs with statistical confidence
- Develop predictive models for estimating urine withdrawal time if these drugs were to be used at extra-label doses
- Provide preliminary data for conducting similar studies for other therapeutic drugs used in show animals

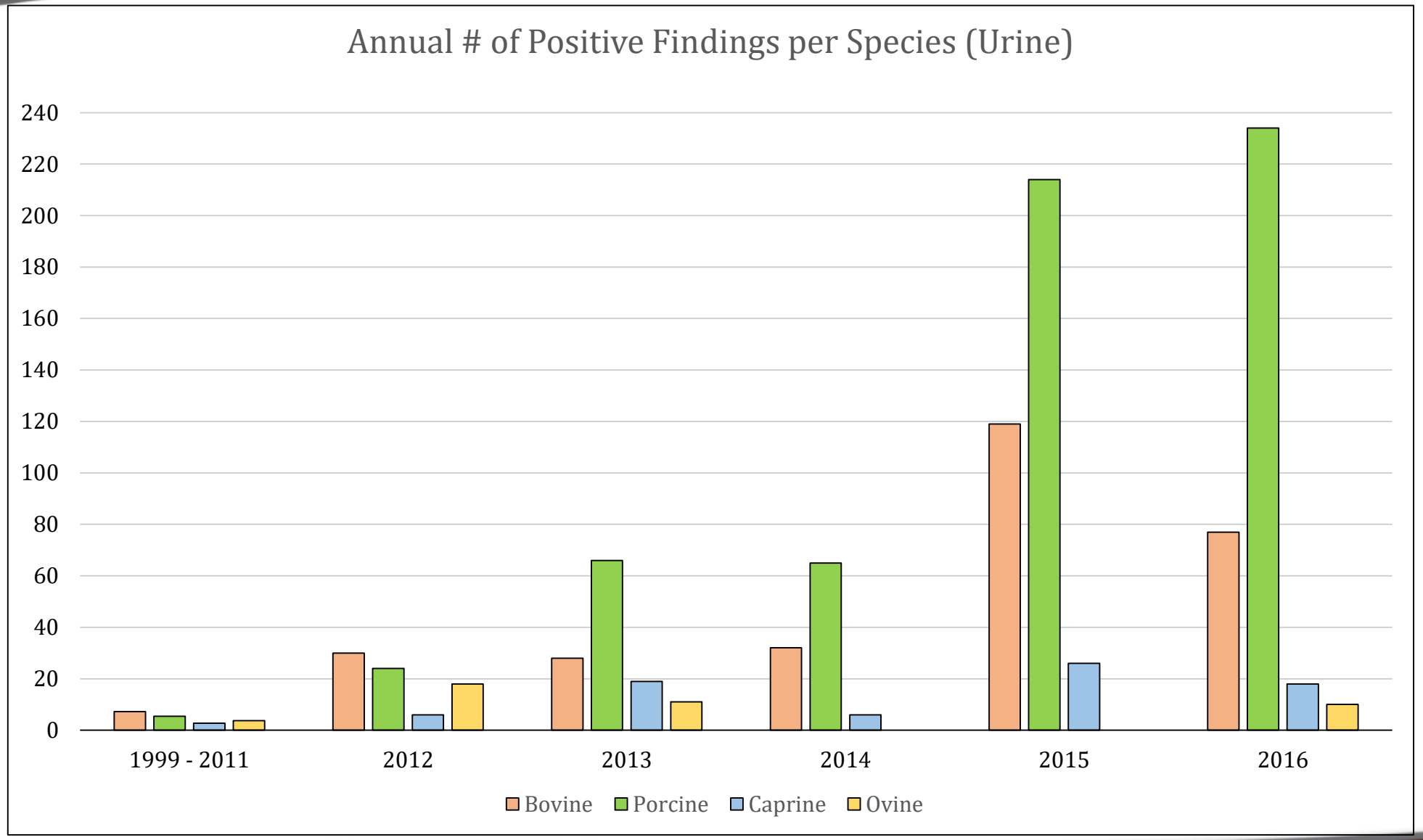
Descriptive Review of Drug Testing Results from 1999 – 2017

Total Number of Shows from 1999 – 2016



Confirmed Drugs in Retina by Species from 1999-2016

	Bovine	Porcine	Ovine	Caprine
Clenbuterol	0	3	6	1
Zilpaterol	2	9	13	5
Ractopamine	1	8	0	2
Total # Tested	24	199	258	284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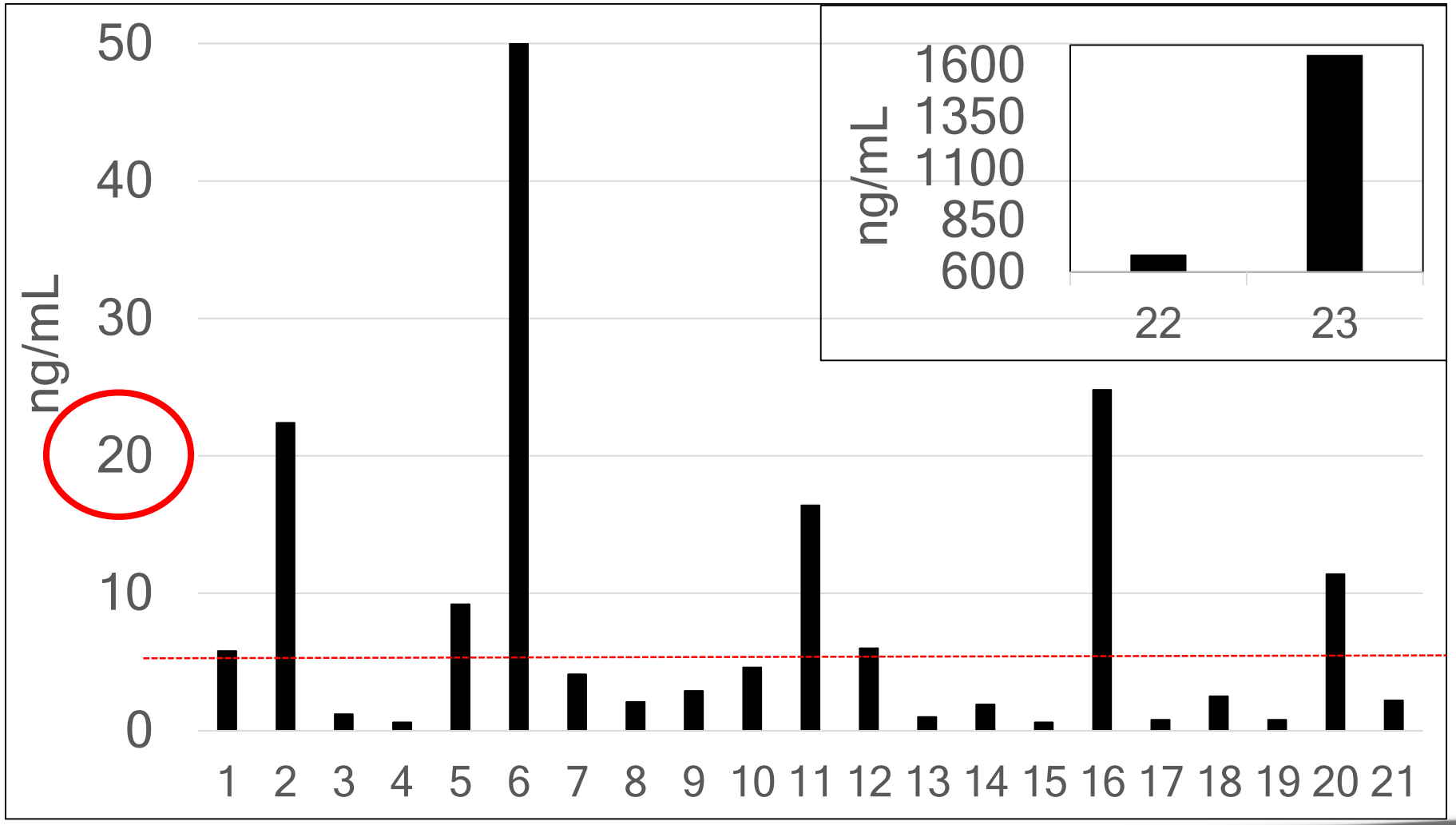
Porcine
Caprine
Ovine

Flunixin Concentrations in Urine (2016-2017)



Hair Testing

Overview

- Broader window of detection compared to serum and urine
- Easy collection and non-invasive
- Potential to estimate time of drug administration
- Currently performed at TVMDL in horses
- Already collected at some shows for DNA testing

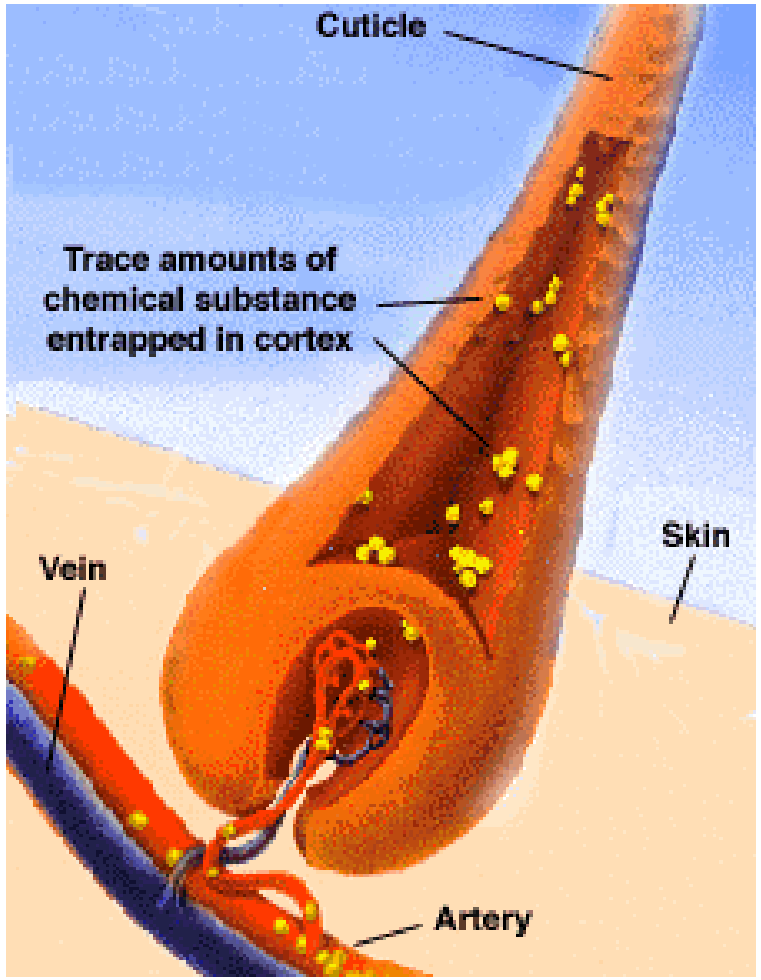


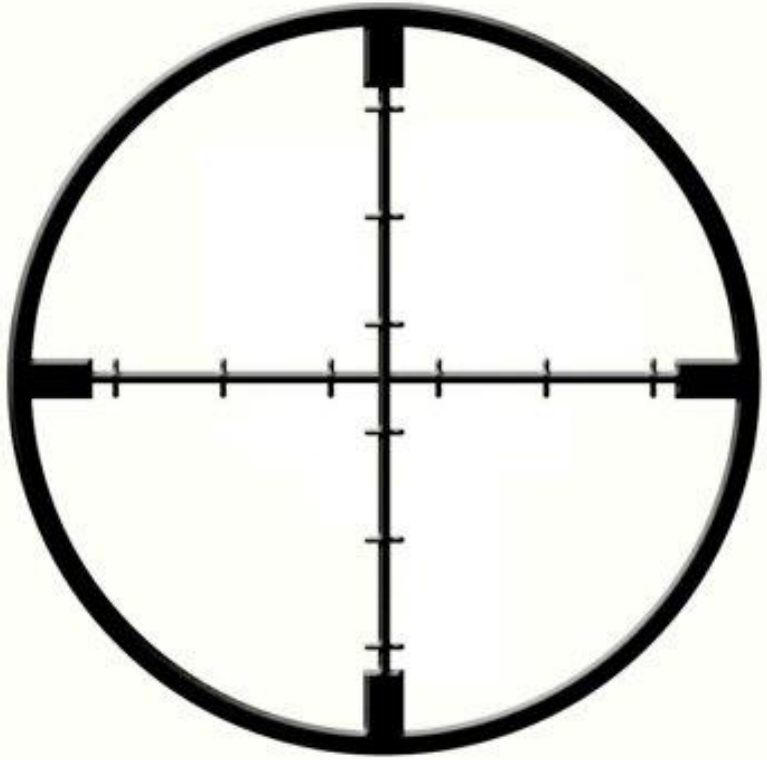
Illustration of Human Hair

Broader Window of Detectability

- Serum and urine are within hours and up to several days
- Some drugs can be detected in hair up to 1 year after administration
- Retina is effective but only available from terminal animals
- Potential for pre-purchase testing

Target Compounds

- Beta-agonists
- Illicit Drugs
- Anabolic Steroids
- Additional growth promoters and repartitioning agents



Need More Information

- Method Validation

- Hair color matters
- Location of hair on body
- Contamination issues
- Rate of hair growth in various species
- Retention time of drugs in hair
- Lack of true positive controls

Summary

- Drug testing is an ever-changing process
  - Improved analytical testing capabilities
  - New drugs and new old drugs
  - New testing strategies and target molecules
- Stay informed of the show's rules/policies
- Work with a veterinarian and keep detailed records
- Research efforts are underway to provide answers and interpretation

QUESTIONS?

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(979) 845-3414

"Aye God, Woodrow, it's been quite a party, ain't it?"