SCIENTIFICALLY VALIDATED REGULATORY THRESHOLDS FOR USE IN RACING REGULATION

BY

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FIRST, A THANK YOU TO RMTC: THE FUROSEMIDE THRESHOLD

First, I am pleased to acknowledge RMTC recognition of this Chay et al 1983 paper supported by HBPA as the scientific basis for the Salix threshold in American racing.

Furosemide	**Chay, S., The pharmacology of furosemide in the horse. V. Pharmacokinetics and blood levels of furosemide after intravenous administration, Drug. Metab. Dispos., 11(3): 226-31 (May/June 1983)**

FUROSEMIDE THRESHOLD: HOW WE GOT THERE

1/ 1982: Kentucky HBPA asked for a **REGULATORY THRESHOLD** to replace the "Detention Barn".

2/ AAEP had identified **Lasix I/V, 250mg/ horse** at **4 hours** prior to post as an appropriate dose/route in prevention of EIPH.

FUROSEMIDE THRESHOLD HISTORY

1/ Dosed 47 horses with AAEP/Dose/ Route/Lasix and quantified plasma furosemide at 1 and 4 hours.

2/ At 1 hour the plasma distribution was “normal” i.e., a bell curve.

2/ At 4 hours the plasma distribution curve was skewed to the right [log normally distributed].

REGULATORY THRESHOLD FOR FUROSEMIDE [1983]

Four hour rule required detention barns. Kentucky HBPA asked us to develop a regulatory threshold, so we dosed 47 horses with 250 mg furosemide I/V.

The figure above right shows the raw data, clearly skewed to the right.

A log transformation normalized this distribution and we estimated that 1/1,000 horses would exceed about 27/30 ng per ML.

Adjusted upward, this became the current regulatory threshold, 100 ng/ML in plasma /serum, linked, courtesy of Dr. Sams, to a 1.010 urinary specific gravity "cut-off".

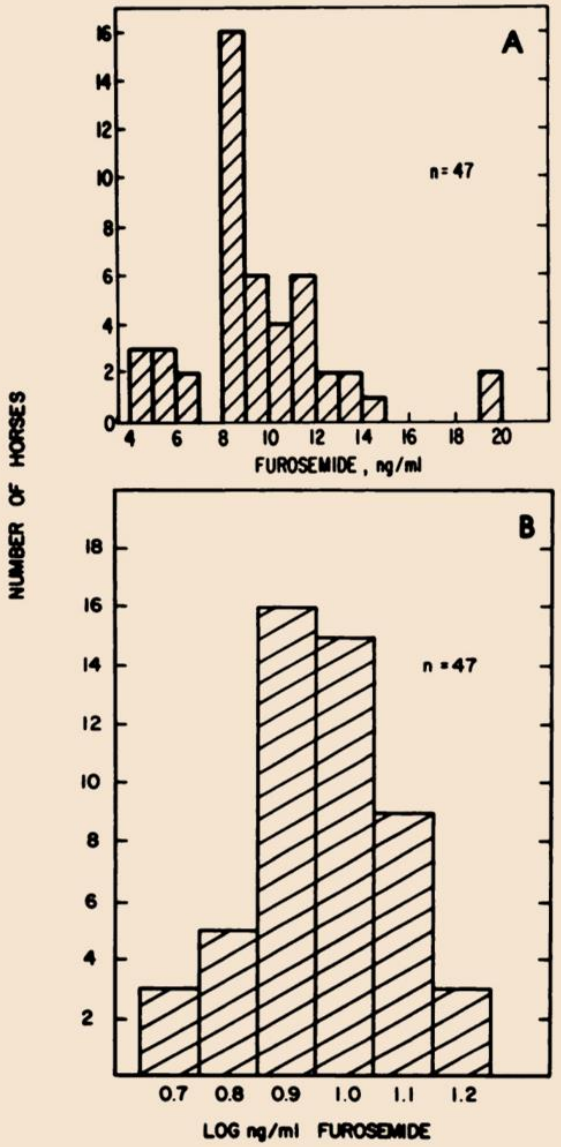


FIG. 6. *Furosemide plasma levels in 47 horses 4 hr after iv administration of 0.5 mg/kg furosemide.*

A, the vertical bars represent the number of horses found within the indicated ranges of furosemide plasma levels; *B*, the vertical bars represent the number of horses found within the indicated ranges of the log of furosemide plasma levels.

FUROSEMIDE THRESHOLD HISTORY

1/ We published these results in 1983.

2/ 1986-7, discussing ELISA testing, Oklahoma Racing Commission asked about Lasix, and they introduced this plasma threshold into Oklahoma racing at I believe, a 60 ng/ml “cut-off”

3/ In retrospect a very useful safety factor.

FUROSEMIDE THRESHOLD HISTORY

1/ In 1998 Dr. Sams suggested adding the 1.010 specific gravity screening level.

2/ Now the RMTC rule, urine specific gravity less than 1.010, plasma furosemide above 100ng/ ml = violation.

3/ This threshold is widely in place in North America.

FUROSEMIDE THRESHOLD HISTORY

This threshold was based on the following:

1.1/ **Defined formulation**, **Specific dose** and a **specific route of administration**.

2/ Administered to a **significant number of animals [47].**

3/ Our **analytical method was capable of quantifying all of the samples.**

4/ Did not have samples that were below our limit of quantification [LOQ] and **we did not eliminate any high samples.**

FUROSEMIDE THRESHOLD HISTORY

1/ Threshold adjusted upward by regulators.

2/ Adjustment allowed threshold to accommodate increases in the furosemide dose to 500 mg/horse by Regulators.

3/ First scientifically based threshold developed and applied in racing chemistry.

GOOD MATH, GOOD THRESHOLD

ACEPROMAZINE

PROPOSED RMTC THRESHOLD FOR ACEPROMAZINE

RMTC proposes a 10ng/ml HEPS regulatory threshold in urine

RCI SCHEDULE OF CONTROLLED THERAPEUTIC SUBSTANCES - Version 1.0
(Adopted April 2, 2013 by Racing Commissioners International.)

Controlled Therapeutic Substance:	Threshold:	No pre-race treatment within:	Dosing Specifications:	Reference Notes	Note:
Acepromazine	10 ng/ml HEPS in urine	48 hours	Single IV dose of acepromazine at 0.05 mg/kg.	UC Davis project	Applicable analyte is metabolite HEPS
Betamethasone	10 pg/mL of plasma or serum.	7 days	IA administration of 9 mg of Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension, USP (American Regent product #0517-0720-01)[i]	RMTC study	IA dosing only - applicable analyte is betamethasone in plasma or serum
Butorphanol	300 ng/mL of total butorphanol in urine or 2 ng/mL of free butorphanol in plasma.	48 hours	Single IV dose of butorphanol as Torbugesic® (butorphanol tartrate) at 0.1 mg/kg.	J. vet. Pharmacol. Therap. doi: 10.1111/j.1365-2885.2012.01385.x	Applicable analytes are total butorphanol (drug and conjugates) in urine and butorphanol in plasma (the drug itself, not any conjugate).

THE RMTC ACEPROMAZINE THRESHOLD

1/ RMTC proposes a 10ng/ml HEPS regulatory threshold in urine.

2/ April 2nd RMTC document cites "UC Davis project"

3/ 10ng/ml HEPS first appeared as an *"in house"* Ohio threshold in 1999, [Tom Journell], less than the previously in place 25ng/ml California threshold.

PUBLISHED RESEARCH BASIS FOR ACEPROMAZINE THRESHOLD [?]

1/ HBPA noted lack of published studies supporting the 24 RMTC thresholds.

2/ RMTC, in a December 4th letter to the Ohio State Racing Commission, listed a number of published studies that they claimed supported their 24 Controlled Therapeutic Medications.

3/ I will now review two of these published papers, one on Acepromazine and one on Phenylbutazone.

December 4, 2013
Mr. Bill Crawford
Executive Director
Ohio Racing Commission
77 South High Street, 18th Floor
Columbus, OH 43215

Issue #8

This allegation is patently false. All substances on the list of 24 have significant scientific research supporting the threshold and withdrawal guidelines. Moreover, the research for these has been vetted by private veterinarians, regulatory veterinarians, analytical chemists, laboratory directors, and veterinary pharmacologists and toxicologists. While the supporting data have not been made public for some of these thresholds, summary reports have been available to the regulatory community prior to adoption of each threshold. As for the list provided by the HBPA, it is also incorrect. Please see the attached list of research by controlled therapeutic medication.

Best regards,
Dionne Benson, DVM
Executive Director

RMTC/RCI Controlled Therapeutic Substances
Reference Chart

Controlled Therapeutic Substance	Reference
Acepromazine	Wieder, M.E., *Identification of acepromazine and its metabolites in horse plasma and urine by LC-MS/MS and accurate mass measurement*, Chromatographia, 75:635-43 (2012)
Betamethasone	HFLSS Study – pre publication
Butorphanol	Knych, H., *Pharmacokinetics and pharmacodynamics of butorphanol following intravenous administration to the horse*, J. vet. Pharmacol. Therap. 36(1):21-30 (Feb. 2013)
Clenbuterol	Knych, H., *Detection, pharmacokinetics and cardiac effects following administration of clenbuterol to exercised horses*, Equine Vet Journal, 2013 June. doi: 10.111/evj.12118 [Epub ahead of print]
Dantrolene	Knych, H., *Pharmacokinetics and metabolism of dantrolene in horses*, J. vet Pharmacol. Therap., 34: 238-46 (June 2011)
Detomidine	L'ami, J.J., *Sublingual administration of detomidine in horses: Sedative effect, analgesia and detection time*, Vet. Journal, 2012 Oct 10. pii: S1090-0233(12)00363-2. doi: 10.1016/j.tvjl.2012.08.016. [Epub ahead of print]

THE WIEDER PAPER:

- Acepromazine as **“Sedalin”** an **oral formulation.**
- Dose; **0.15 mg/kg, three times** the RMTC IV dose.
- **Number of horses six**, a very small number for a threshold study.
- Nowhere in the sdudy can I find a regulatory threshold or a withdrawal time guideline for acepromazine.

THE WIEDER ACEPROMAZINE PAPER

I quote from the Wieder study, page 641:

"Once again it must be noted that the windows of quantification are derived from use of a highly sensitive targeted method Therefore, they do not necessarily reflect a routine screening situation and consequently do not reflect detection time advice that may be offered by official regulatory bodies."

NO MATH, NO THRESHOLD

PHENYLBUTAZONE

RMTC CITED PHENYLBUTAZONE STUDY

Phenylbutazone	Chay, S., Population distributions of phenylbutazone and oxyphenbutazone after oral and i.v. dosing in horses, Am. J. Vet. Res., 67(4): 654-62 (Dec. 1984)

1/ This 30 year old paper cited by the RMTC in support of the phenylbutazone threshold is authored by Soma and Tobin, among others.

2/ However, RMTC chooses to ignore the RMTC phenylbutazone study carried out in Florida 26 years later, in or about 2010, which samples were analyzed in Pennsylvania and Florida.

RMTC BUTE PA ANALYSIS

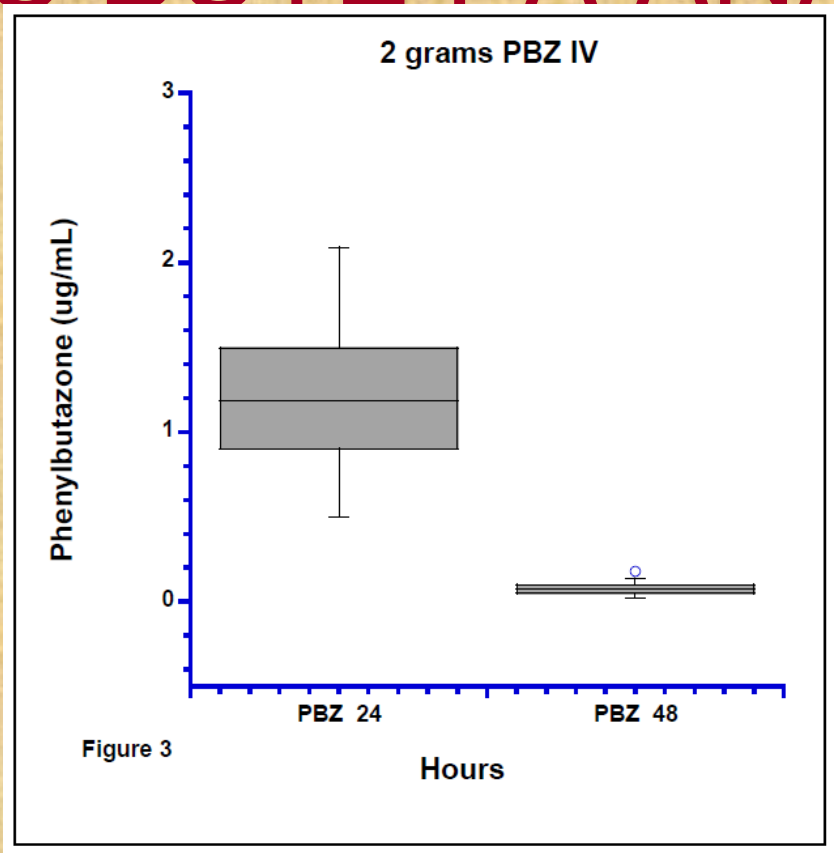


Figure 3

These data indicate that even with no prior administrations of PBZ a dose of 2 grams 24 hours prior to post-time may produce a violation of the 2 µg/ml threshold. It is suggested that if a dose of 4.4 mg/kg is to be administered that the weight of the horse be taken into consideration or PBZ be administered at 36 hours prior to race time.

RMTC BUTE PA ANALYSIS

Figure 3 shows the results of a single IV administration of 2 grams (4.4mg/kg) of phenylbutazone (PBZ). This group of 20 horses did not have prior administration of PBZ.

The 24 hour mean post-administration plasma concentration was 1.2 **(range of 0.5 to 2.1) µg/ml** and mean 48 hours plasma concentration was 0.08 (range of 0.02 to 0.18) µg/ml.

These data indicate that even with no prior administrations of PBZ a dose of 2 grams 24 hours prior to post-time may produce a violation of the 2 µg/ml threshold. It is suggested that if a dose of 4.4 mg/kg is to be administered that the weight of the horse be taken into consideration or PBZ be administered at 36 hours prior to race time.

Phenylbutazone IV administration to 20 horses (courtesy of the Drs Sams and Callahan of the University of Florida).

MATH SAYS DANGEROUS THRESHOLD [and PA]

THE RMTC CITED PAPERS:

Presented the Wieder study as supporting the RMTC acepromazine threshold/ withdrawal time guideline has no scientific basis or validity whatsoever.

Presented a 30 year old phenylbutazone study and ignored a much more recent 2010 RMTC study. An attempt to draw attention a away from the more recent RMTC study?

THE 2UG/ML PHENYLBUTAZONE THRESHOLD

HOW DANGEROUS?

AN ANALYSIS

RMTC PHENYLBUTAZONE STUDY

20 horses in training, dosed 2g IV, plasma and serum samples taken, late May, 2010.

Prompt analysis in Pennsylvania, later analysis in Florida.

September 10th, Pennsylvania suggests treat as 36 hour rule or reduce the “24hr.” dose.

2UG/ML PHENYLBUTAZONE Raw data

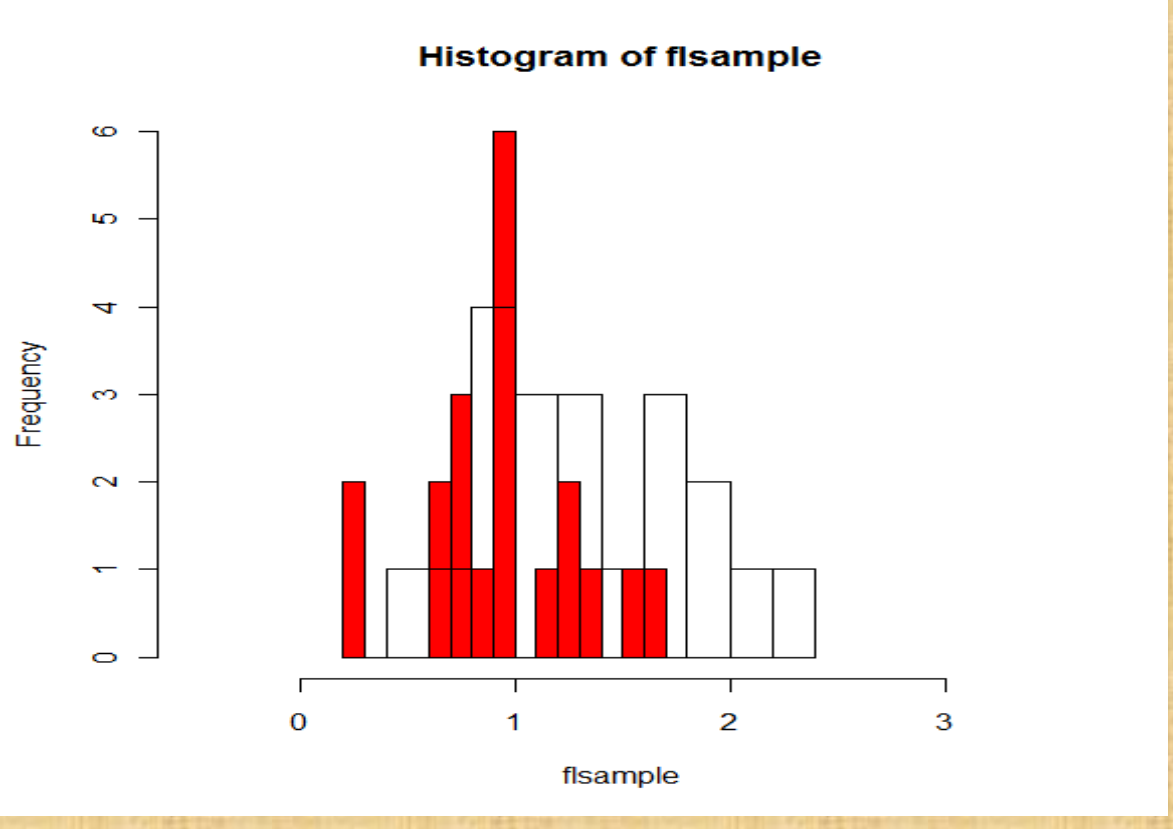
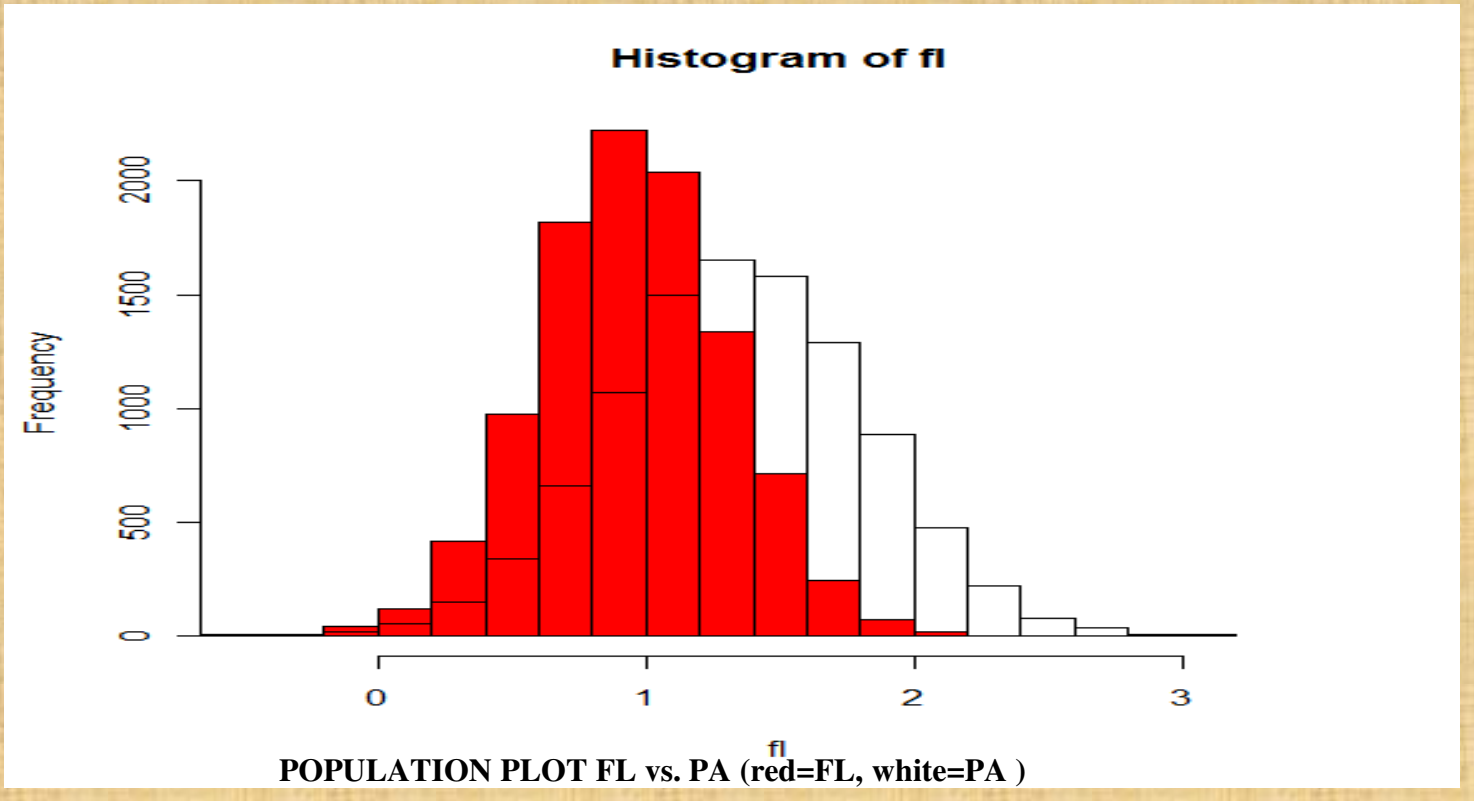


Figure #1: Serum phenylbutazone concentrations sample histograms, Florida samples in red vs Pennsylvania samples in white. (red=fl, white=pa

2UG/ML PHENYLBUTAZONE Statistical Projections



POPULATION PLOT FL vs. PA (red=FL, white=PA)

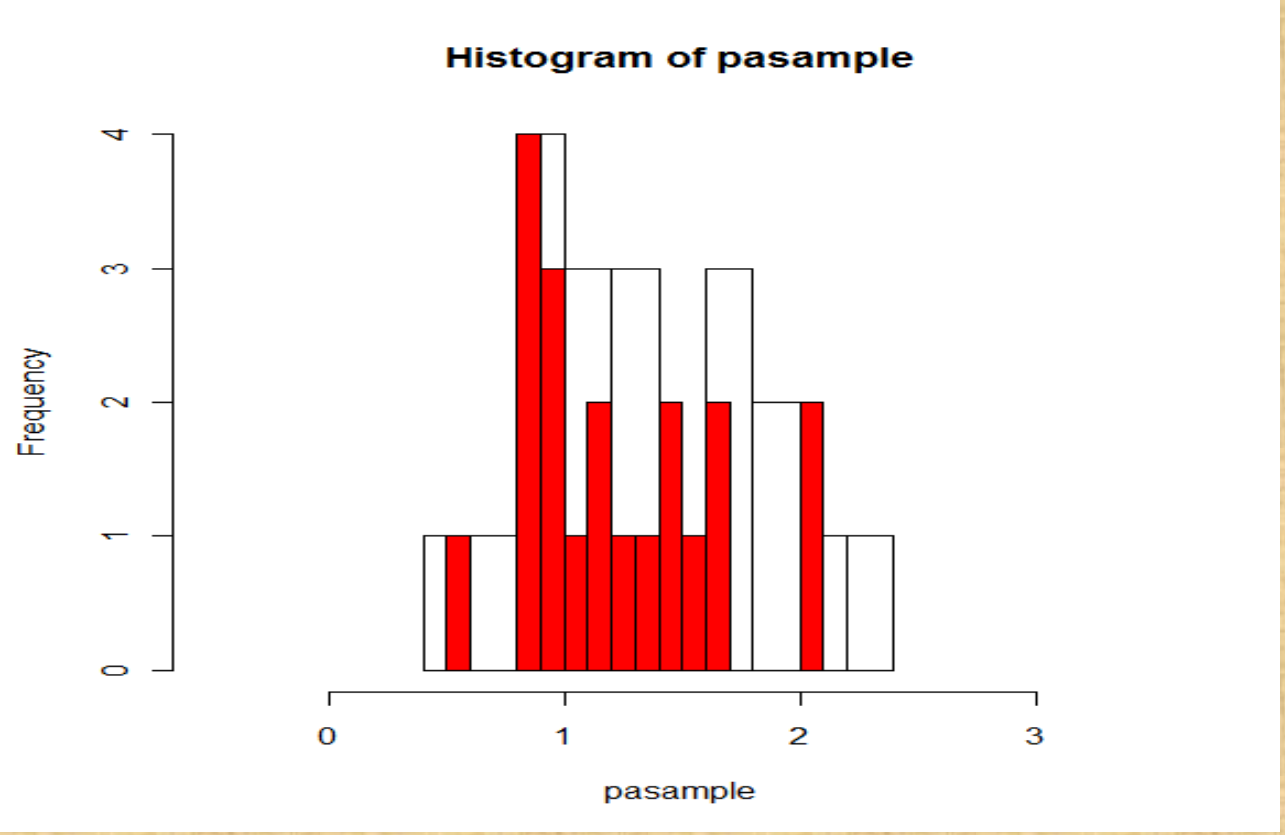
# horses out of 17,500	2~3(ug/ml)	3~4(ug/ml)	4~5(ug/ml)	>5(ug/ml)
PA	1,400	3.5	0	0
FL	30.8	0	0	0

2 ug/ml TAKE HOME MESSAGE

Pennsylvania analysis of RMTC data suggests about an 8% overage rate.

This is consistent with the University of Pennsylvania advisory to Pennsylvania Horsemen suggesting that the 2 ug/ml threshold be treated as a 36 hour with-drawal time.

PLASMA /SERUM DIFFERENCES



PA Sample histogram serum vs. plasma (red=plasma white=serum)

SAMPLE	Mean (ug/ml)	Sd(ug/ml)
PA serum	1.343	0.468
PA plasma	1.237	0.4227

2UG/ML PHENYLBUTAZONE

2 ug/ml ? How does a 2 ug/ml threshold relate to a 24 hour withdrawal?

NOT WELL: Even with complete compliance there will be “random” overages.

Plan for approximately 10% overages, at least

Pennsylvania: A 36 hour rule, or reduce the dose

TAKE HOME MESSAGE

Ronald Reagen: “Trust but Verify”

Verify: Ask for the Math; if they can show you data points and a valid statistical probability, then you know as exactly as you can where you are.

If not, where you are is likely going to be where they choose to put you.

Acknowledgements

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2UG/ML PHENYLBUTAZONE

The 24 hour threshold for phenylbutazone has long been 5 ug/ml.

2 ug/ml ? How does a 2 ug/ml threshold relate to a 24 hour withdrawal?

NOT WELL: Even with complete compliance there will be “random” overages.

Plan for approximately 10% overages, at least

Pennsylvania: A 36 hour rule, or reduce the dose