

ELISA TESTING OF RACING HORSES: A REGULATORY REVOLUTION

By
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NEOGEN AND FORENSIC CHEMISTRY

- Neogen has available a large and expanding range of high quality forensic tests
- Largest selection of high quality forensic ELISA tests available worldwide
- Available to Equine and Human Forensic Labs.

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HISTORY OF DRUGS IN HORSES

- The "less than proper" medication of racing horses has a long history, with an absolutely impeccable starting point, **Greek mythology**

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Greek Mythology

DIOMEDES

Diomedes fed his mares human flesh to make them savage and unbeatable. (Name of his veterinarian not recorded)

These activities were not well regarded by the Greek Jockey Club.

Greek regulatory chief had an outstanding professional reputation in antiquity.

His name was HERCULES.

(Name of Forensic Chemist not recorded; SOP not available)

Diomedes was disciplined severely by Hercules. (Note the large club)

Artwork: Courtesy of Charles Le Brun, Painter to the King of France, circa 1645



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Racing, the Sport of Kings:



Philip of Macedon (Father of Alexander the Great) entered a horse in Greek Olympics. (Note: unusual for a non-Greek to be allowed to enter the Olympics)

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Quality Assurance



His horse won the race. Philip struck a commemorative coin. The chisel mark on this coin records it being presented in a Greek marketplace, challenged, and then tested for authenticity (Quality Assurance) by the official Greek market assayer.

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France, use of medication circa 1810. Artwork courtesy of Carl Vernet.

HORSERACING

Horsemen always at the cutting edge

Horseracing, and especially American horseracing, has a long and not insignificant history of exploring drug and medication use.

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How do Some Horsemen try to Win Races?

Any way they can, including but not limited to the creative use of drugs and medications

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Acute Stimulant Medication

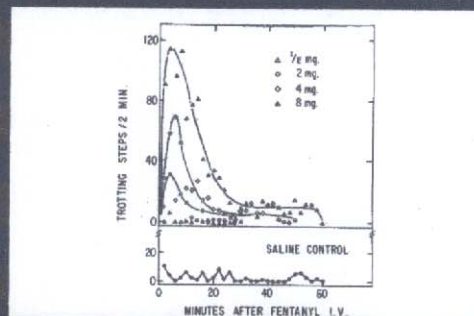
- **Classic stimulants:** cocaine, caffeine, amphetamine, methylphenidate, methamphetamine, mazindol
- **Opiates:** etorphine, fentanyl, morphine, buprenorphine
- **Tranquilizers:** "washy horses": acepromazine, promazine, fluphenazine, detomidine, etc.
- **Bronchodilators:** clenbuterol, terbutaline, atropine, ipratropium bromide
- Always illegal and usually an "inside job"

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Narcotic Analgesics

- Morphine/synthetic narcotic analgesics
- Locomotor stimulant
- Suppress pain
- Prolongs endurance
- Potentiated by stimulants
- Reliable, classic stimulant medication
- Widely used pre-ELISA

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Dose response curves for the locomotor stimulant effects of fentanyl, administered by IV injection at the indicated doses

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Bronchodilators

- Beta-2 agonist bronchodilators: clenbuterol, albuterol, terbutaline, etc.
- Highly potent agents
- Presumably improve oxygen delivery to blood and musculature
- Widely used close to post prior to ELISA
- Post-ELISA: use cut back dramatically

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Using Tranquilizers to Improve Performance

- Tranquilizers can be used to treat "washy" horses, that is horses that "run their race in the paddock". The net result is an improvement in performance.
- Allows jockey to "rate" horse in the race.
- Detomidine, reserpine, acepromazine, promazine.
- All legitimate therapeutic agents used in veterinary practice: rate of improper use??

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Chronic Stimulant Medication

- Anabolic steroids, growth hormone, erythropoietin (EPO)
- Special food supplements
- Vitamins
- By definition an "inside job"
- With the exception of anabolic steroids, not currently a very active area

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"Stopping" Horses

- "Nobbling" the English slang term
- Horses heavily backed "ante-post"
- Administer tranquilizer or depressant to slow or stop a favorite
- Oldest form of illegal medication
- Usually considered an "outside" job

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"Getting at a horse"

"Nobbling"

Always illegal
and in this case
seems to be an
"outside job"



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Medication to Lose

- The oldest form of illegal medication
- In principle, always possible to medicate a horse so that it will lose
- Challenge is to treat a horse such that he goes to post but does not win his race
- More likely where there is heavy ante-post betting
- Also possible to medicate a horse to lose so that a favored horse will win
- Always illegal, and usually considered an "outside" job

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Medication to Restore "Normal" Performance

- Thought is that you can medicate a horse to restore "normal" performance
- No stimulant or depressant actions
- Non-steroidal anti-inflammatory agents (NSAIDs)
- Prophylaxis of EIPH: furosemide
- Corticosteroids?

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Medication to "Mask" Other Agents

- Administer agents that will interfere with testing
- As testing improves, a less likely scenario
- Furosemide: dilution
- Polyethylene glycol: smear TLC plates
- Thiamine: interfere with UV analysis

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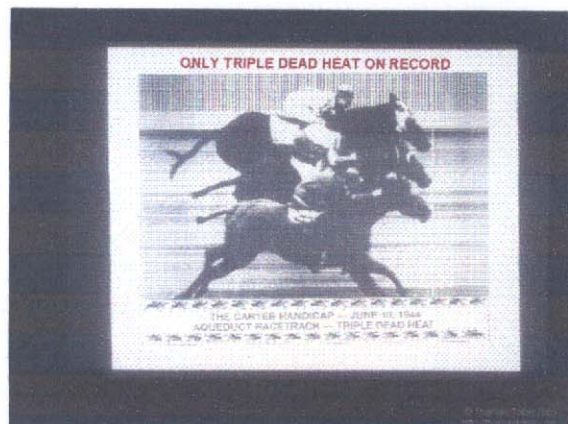
Medication to Dilute Out Other Drugs

- Administer a drug that dilutes drugs/drug metabolites and makes them more difficult to detect
- Diuretics generally do not affect blood levels of drugs
- Dilute out water soluble drugs/drug metabolites in equine urine
- Effect is generally up to but not greater than the diluting effect
- Rules on furosemide in the US specify dose and time of administration

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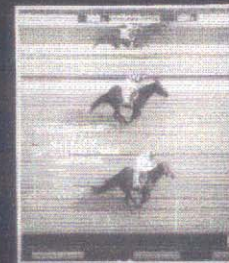
Can Drugs/Medications Influence Racing Performance?

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Grindstone wins the
1996 Kentucky Derby



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Secretariat wins the Belmont

Equine Drug Testing

Equine Drug Testing

- Equine drug testing has been in place longer (since 1910), and is more rigorous and extensive (250-2,500 agents) than any other ongoing human drug testing endeavor
- **WHAT HAPPENS**
- Horsemen use drugs → Chemists develop tests → Horsemen switch to new drugs → Chemists develop new tests; one outcomes is that:
- **Neogen has a large and expanding panel of Forensic Drug Tests**
ad infinitum?

Outcome for Forensic Chemists:

Neogen has available a large and expanding range of high quality forensic ELISA tests

-AD INFINITUM?-

Equine Drug Testing

- Based on post-race urine samples
- About 10% of runners sampled/500,000 samples/year worldwide
- Winners, beaten favorites, selected horses that the stewards nominate
- Usually a central University-based laboratory, testing 10-40K samples/year
- Scope of testing very/extremely broad: 250-2,500 agents

EQUINE DRUG TESTING

1985

Equine drug screening based on Thin Layer Chromatography

Limited Sensitivity:

High-potency narcotics & stimulants were being used with **relative impunity.**

High Potency Agents

- Any drug given to a horse < 10 mg/horse
 - Etorphine, Fentanyl, Acepromazine, Mazindol, Terbutaline, Buprenorphine, Cocaine, Oxymorphone, Detomidine
- Not being reliably detected pre-ELISA
- Being used at will by some horsemen

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Summer, 1985:

TT Received a
“Directive from On
High (Chair of the
Kentucky Racing
Commission)”

“Fix the problem”

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The 1980's: ELISA Testing

- Immunoassay program starts: Illinois Racing Board Laboratory, IDS, a Biotech Company, and the University of Kentucky
- Sensitivity of testing increased 100-1,000 fold, virtually overnight, for 20+ drugs
- Multiple identifications of narcotics, stimulants, tranquilizers, bronchodilators

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ELISA Testing

- Enzyme Linked ImmunoSorbent Assay (ELISA)
- Simple, fast, high sensitivity testing
- ~1,000 times more sensitive than thin layer chromatography
- In 1985, UK started a research project that rapidly evolved into the ELISA testing program

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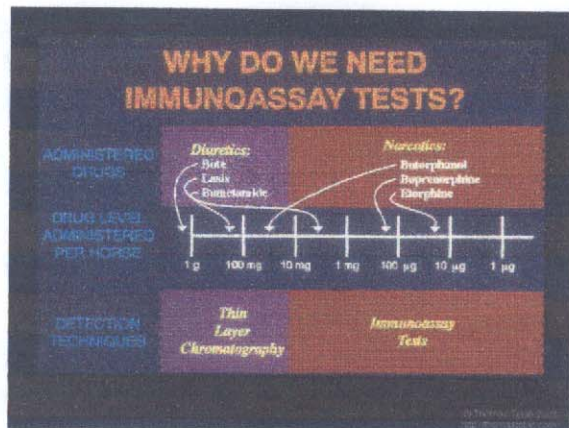
Enzyme-Linked ImmunoSorbent Assay (ELISA) Testing

Thomas Tobin, Daniel Tai, and David Watt, 1985-1992

- 1985: Kentucky Racing Commission requests control of high potency narcotics
- 1986: RIA tests for fentanyl and etorphine (“Elephant juice”)
- 1987: Program develops ELISA tests
- 1988: ELISA tests dramatically effective: POLITICS
- 1989: Program reorganized – development of tests continues
- 1992: 50 ELISA tests for high potency drugs, yielding ELISA Technologies, a division of Neogen Corporation

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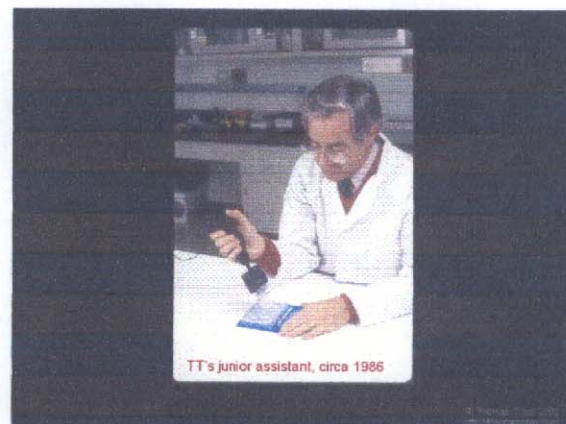
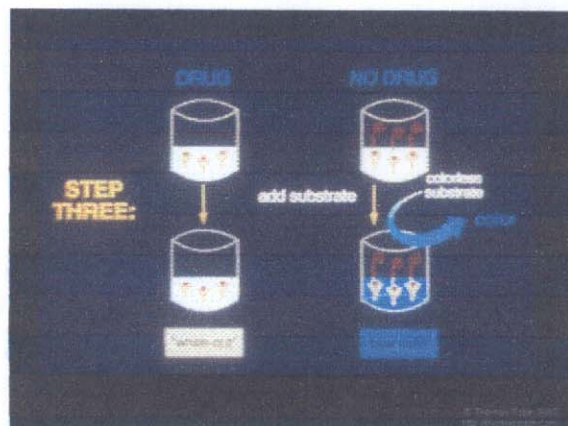
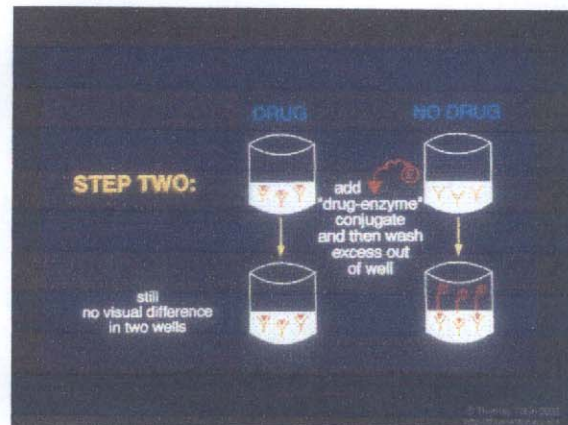
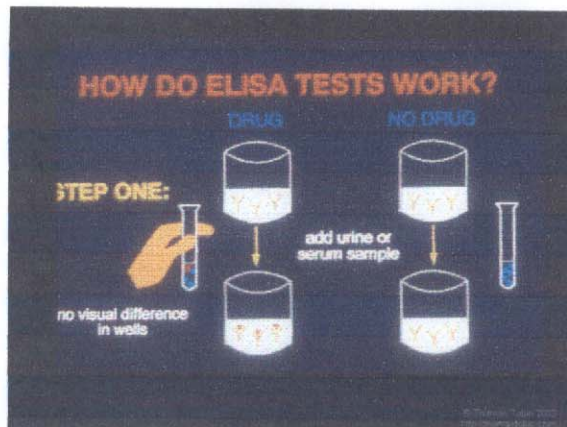


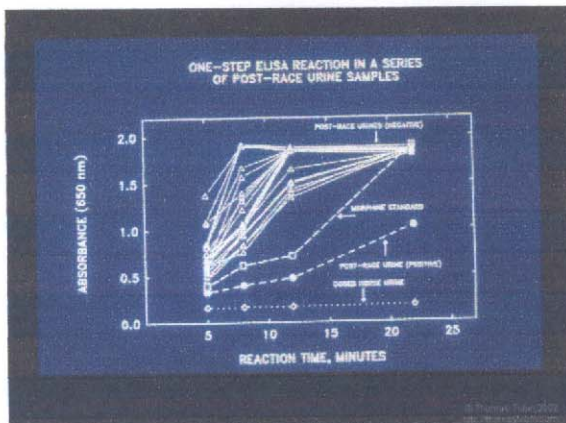
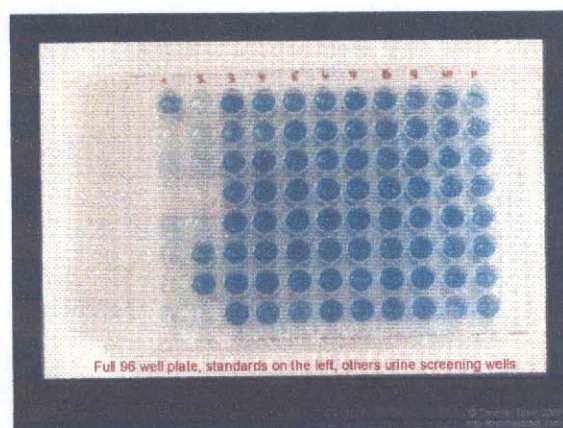


How to make an ELISA test

- Obtain drug or drug structure
- **Derivatize drug: Insert COOH or NH₂ group: hapten**
- Link derivatized drug (hapten) to BSA
- Inject into rabbit, re-inject, wait,
- Link hapten to Horse Radish Peroxidase (HRP)
- Check rabbit serum for antibodies
- Construct and validate ELISA test

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ELISA Screening of Post-Race Urine Samples Followed by Mass Spectral Analysis

Sample Date	# Urine Samples	# Flagged by ELISA	# Positive by GC/MS analysis	Drug Identified
10-3,4-87	34	5	3	Oxymorphone
10-4-87	16	1	1	Oxymorphone
10-11-87	8	1	1	Oxymorphone
10-17-87	36	3	2	Oxymorphone
10-17,18-87	27	3	1	Oxymorphone
10-20-87	21	4	4	Oxymorphone
10-27-87	24	1	1	Hydromorphone
Totals, 9 Days	166	18	13	

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Efficacy of PCFIA/ELISA Tests, 1987-1988

Drug	State	TLC Status	Immunoassay Positives
Buprenorphine	New Mexico	No test	Multiple (>50)
Oxymorphone	New Mexico	Poor sensitivity	Multiple (>30)
Sufentanil	Oklahoma	No test	10/300*
Mazindol	Western states	Very poor sensitivity	Multiple (>20)
Cocaine	California	Poor sensitivity	2/83*
Acepromazine	Illinois	Fair sensitivity	Multiple (>25)**

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Fall 1987

- ELISA testing of **frozen post-race samples** in New Mexico
- First panel of ELISA tests puts down 40 plus trainers in New Mexico
- A problem that had been in place, in one form or another for 100 years, had been controlled

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The 1980's: Impact of ELISA Testing

- Probably the biggest single "across the board" increase in the sensitivity of equine drug screening
- Stopped patterns of medication that had been in place in one form or another for the better part of 100 years
- Also greatly increased the sensitivity of testing for therapeutic medications

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ELISA Testing

- Offers virtually complete control of the abuse of high potency medications
- Also offers super-sensitive testing for therapeutic medications
- Has accentuated a long-standing problem in equine medication control: Detection of trace residues of therapeutic medications and environmental and endogenous substances

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Where To Go, 1987?

- 1987 -- Five ELISA tests (but 100's of drugs)
- 2002 -- Neogen's nearly 100 ELISAs, can screen for more than 250 agents
- Many current ELISA tests are of interest in **HUMAN FORENSICS** and the **FOOD SAFETY INDUSTRIES**

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Neogen's ELISA Tests for Use in Racing (May 1st, 2002)

- Alfentanil, amphetamine, amphetamine ultra, anileridine, apomorphine, azaperone, barbiturate group, benzodiazepine group, boldenone, bronchodilator group, bumetanide, buprenorphine, butorphanol, caffeine/pentoxifylline, carfentanil, clenbuterol, cocaine/benzoylcegonine, corticosteroid group, cromoglycate, dantrolene, detomidine, dexamethasone, diprenorphine, doxapram, droperidol, ethacrynic acid, etorphine, fentanyl group, fentanyl, flunitrazepam, flunixin, fluoxetine, fluphenazine, furosemide, glycopyrrolate, guanabenz, haloperidol...

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Neogen's ELISA Tests, cont'd

- Haloperidol metabolite, hydromorphone, isoxsuprine, ketamine, ketorolac, levallorphan, lidocaine, lofentanil, mazindol, mazindol metabolite, meperidine, mephentermine, mepivacaine, methadone, methamphetamine/MDMA, methocarbamol, methotrexate, methylphenidate, methylprednisolone, nalbuphine, nandrolone, opiate group, oxymorphone/oxycodone, pentazocine, phenylbutazone, procaine, promazine group, propranolol, pyrilamine, reserpine, stanozolol, sufentanil, sulfamethazine, THC metabolite, theophylline, trenbolone, triamcinolone acetonide, tricyclics group, zomepirac

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Neogen's Forensic Market ELISA Tests

Alfentanil, Amphetamine, Barbiturate group, Benzodiazepine group, Boldenone, Buprenorphine, Butorphanol, Carfentanil, Cocaine/BZE, Etorphine, Fentanyl group, Flunitrazepam, Hydromorphone, Lofentanil, Ketamine, LSD, Meperidine, Methadone, Methylphenidate, Methamphetamine/MDMA, Nandrolone, Opiate group, Oxymorphone/Oxycodone, Sufentanil, THC Metabolite

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NEOGEN AND FORENSIC CHEMISTRY

- Neogen has available a large and expanding range of high quality forensic tests
- Largest selection of high quality forensic ELISA tests available worldwide
- Available to Equine and Human Forensic Labs.

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Efficacy of Equine Drug Testing

- Rate of detection of performance altering agents in racing is relatively low
- "Positives" in about 1-1,000 samples tested.
- All identifications confirmed by mass spectrometry in the primary and the referee laboratory.
- "Top-end" of racing is actually very clean
- Thanks in part to the technology I have just described

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SUMMARY

- ELISA testing technology is highly effective: the Worldwide Standard in Racing
- Simple, fast, inexpensive and highly sensitive
- Technology is highly adaptable
- Single, portable "Horse Side" tests
- Inexpensive automated systems
- High end, high volume, high throughput completely automated systems

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THREE CUSTOMIZED LEVELS OF ELISA TEST UTILIZATION

- | | |
|-----------------------|---|
| • Limited Throughput: | 10K samples/year
5-10 tests/sample |
| • Medium Throughput: | 20K samples/year
10-15 tests/sample |
| • High Throughput: | 100K samples/year
25-30 tests/sample |

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LIMITED VOLUME THROUGHPUT

- Contract to buy a certain annual volume of ELISA tests (100K tests/year, or so)
Make available fully automated \$50K
- Thermolabsystems 'DSX' Analyzer to perform the ELISA analyses

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Thermolabsystems 'DSX' Analyzer

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Thermolabsystems 'DSX' Analyzer

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MEDIUM VOLUME THROUGHPUT

- Contract to buy a certain annual volume of ELISA tests (300K tests/year, or so)
- Make available fully automated \$100K **Beckman TECAN Robotic System** to perform the ELISA analyses
- Information:
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Beckman TECAN Robotic System

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HIGH VOLUME THROUGHPUT

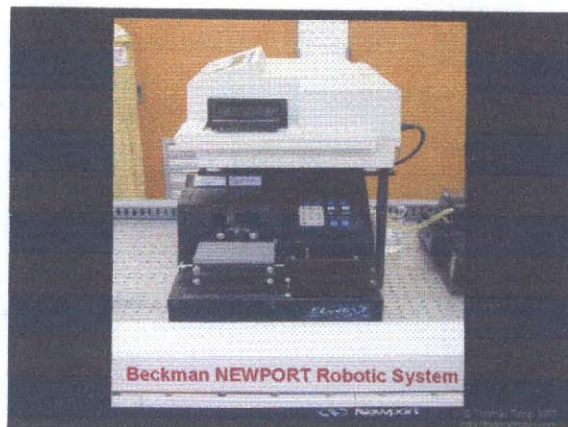
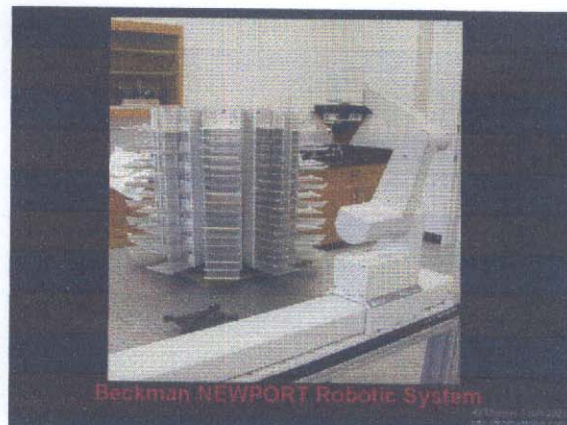
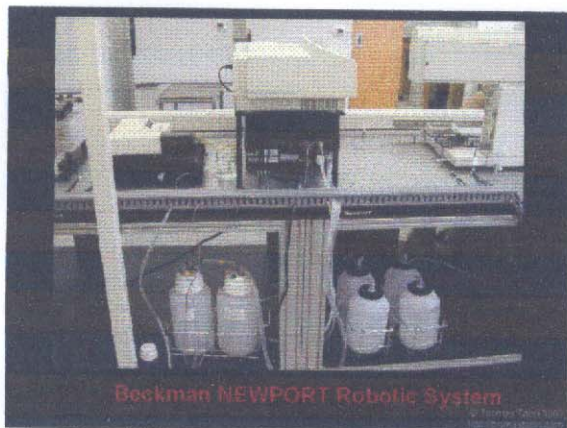
- Contract to buy a certain annual volume of ELISA tests (3,000K tests/year, or so)
- Make available fully automated \$200K **Beckman NEWPORT Robotic System** to perform the ELISA analyses
- Information:
scottcongleton@Neogen.com

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Beckman NEWPORT Robotic System

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THANKS TO THE FOLLOWING

- Thanks to my longtime colleagues at Neogen Corp, for their ongoing support in expanding and commercializing this technology
- To all the members of my research group, and especially Drs. W. Karpiesiuk, S. Kwiatkowski and Mr. W. E. Woods.
- The Kentucky Racing Commission and the Kentucky Equine Drug Council for support
- To the Director of the Gluck Equine Research Center and to my colleagues there and at UK for their support over the years

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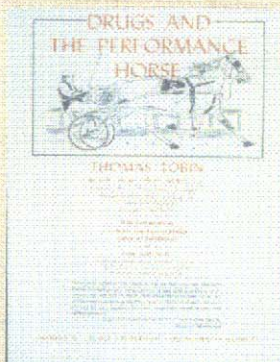
Finally, in closing, thank you
very much, Ladies and
Gentleman, for your kind
attention this morning

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LITERATURE REFERENCES

- 1/ Remifentanyl in the Horse: Identification and Detection of its Major Urinary Metabolite; Lehner et al., *J. Anal. Tox.* Vol 24, July/August, 2000
- 2/ "Kentucky Laboratory Takes Lead Against Horse Dopers," Tobin, *Clinical Chemistry News*, Vol. 18, Issue 1, January 1992
- 3/ Non-isotopic Immunoassay Drug Tests in Racing Horses: A Review of their Application to Pre- and Post-Race Testing, Drug Quantitation and Human Testing; Tobin et al., *Res. Comm. Chem. Path. Pharmacol.* Vol 62, #3, P371-396, 1988

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