Cleveland Clinic

Evaluation of a Centrifugal Filtration Device in the Measurement of Free Testosterone

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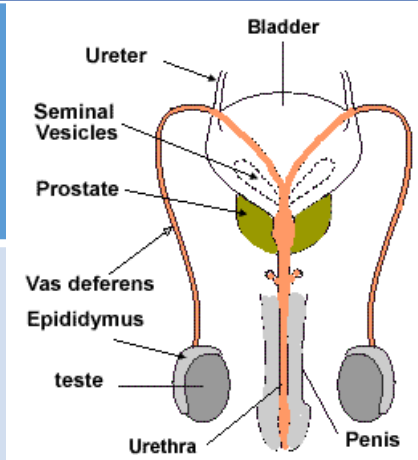
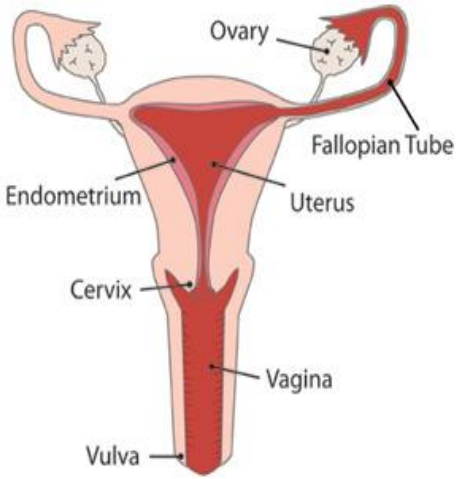
Cleveland Clinic Background

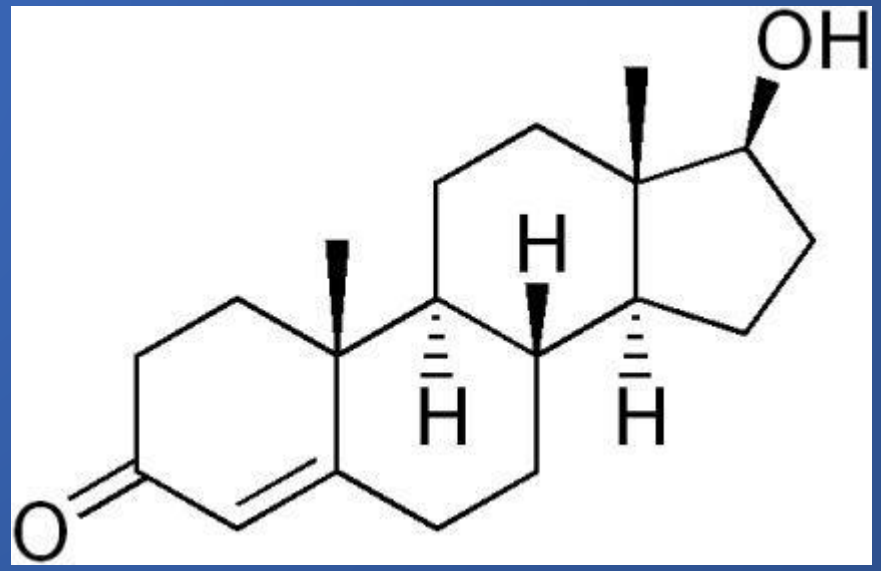
- Testosterone
  - 2014 annual tests - 28,200
  - Automation (Enzyme Immunoassay)
- Free testosterone
  - 2014 annual tests - 21,000
  - Indirect measurement
  - Manual (RadioImmunoassay)
  - Ultrafiltration
    - Millipore Centrifree™ Ultrafiltration device

Testosterone

	Male		Female	
Produced	• Testes • Adrenal glands		• Ovaries • Adrenal glands	
Responsibility	• Development of external genitalia • Secondary sex characteristics		• Estrogen precursor • Hormonal balance	
Why	• Infertility (low sperm counts) • Sexual problems (decreased sex drive/erectile dysfunction) • Premature/delayed puberty • Osteoporosis • Monitoring of testosterone therapy		• Irregular menstrual cycles • Development of male features (hirsutism)	

Total vs Free Testosterone

- Total testosterone = bound + free testosterone
- Majority of testosterone is bound
  - Sex hormone binding globulin (SHBG)
  - Albumin
- Free Testosterone
  - Unbound



Free Testosterone

- Metabolically active
- SHBG driven
  - Increase in SHBG = Decrease in Free Testosterone
  - Decrease in SHBG = Increase in Free Testosterone
- Testing importance
  - Androgen deficiency in males
    - Hypogonadism
  - Androgen excess in females
    - Polycystic Ovary Syndrome
    - Hirsutism

Free Testosterone Separation

Two methods of separation

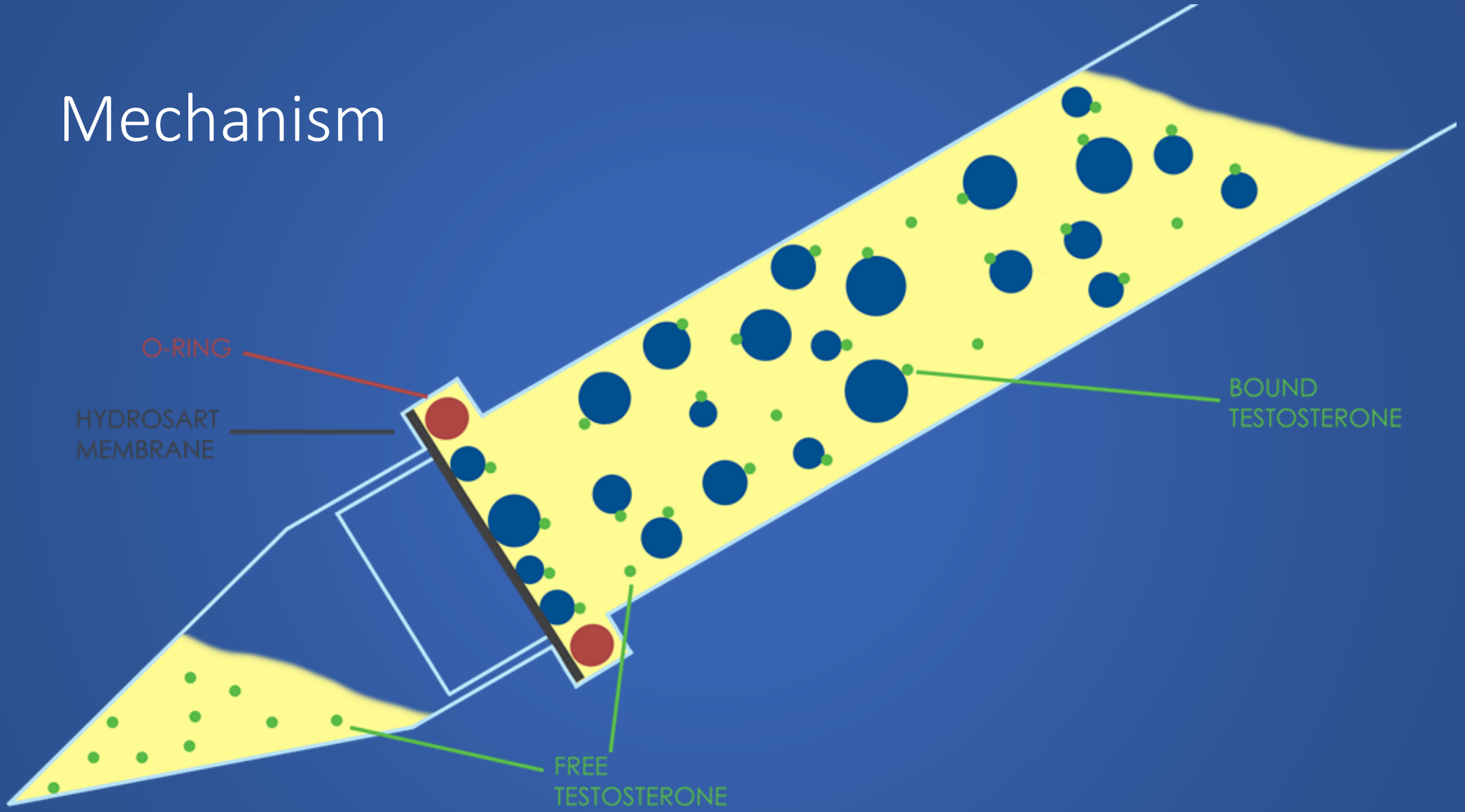
- Equilibrium Dialysis (gold standard)
  - Time consuming
  - Technically difficult
  - Error prone
  - Not suitable for high volume testing
- Ultrafiltration
  - Faster
  - Technically easier
  - Centrifuge limitation

Ultrafiltration Device Evaluation

- Centrifree™ (EMD Millipore)
  - Current ultrafiltration device in use
  - No quality/testing issues with the device

- Vivafree™ (VivaProducts)
  - Proposed ultrafiltration device
  - Filter based on molecular weight of 30,000 daltons

Mechanism

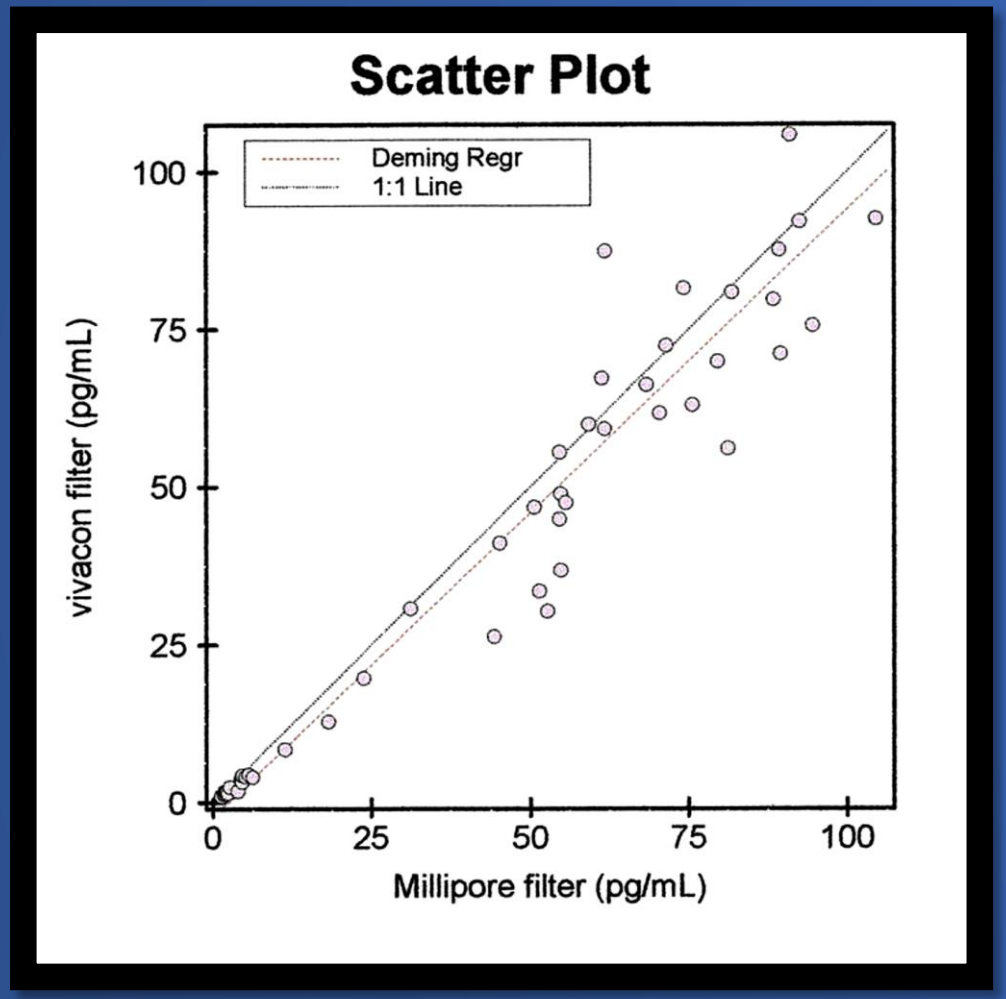

O-RING
HYDROSART MEMBRANE
BOUND TESTOSTERONE
FREE TESTOSTERONE

Free Testosterone Comparison

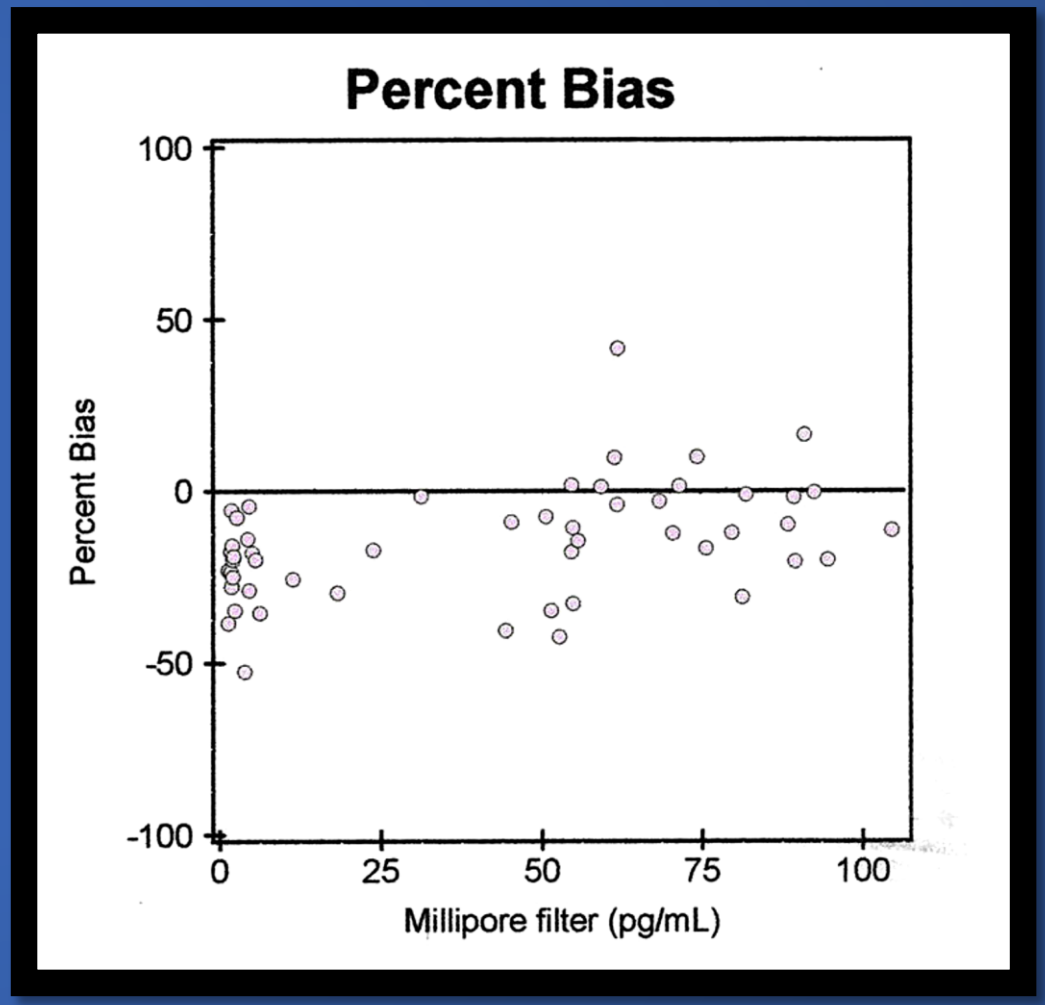
- 53 serum samples
- Filtrates measured by RadioImmunoassay

Sample Size	300 µL
Centrifuge Speed	4500 rpm
Centrifuge Time	15 min
Filtrate sample size	100 µL

Comparison Results -15 min spin



Slope = 0.963
Corr Coef (R) = 0.9685



Average % Bias = -9.71

Intraday Precision -15 min spin

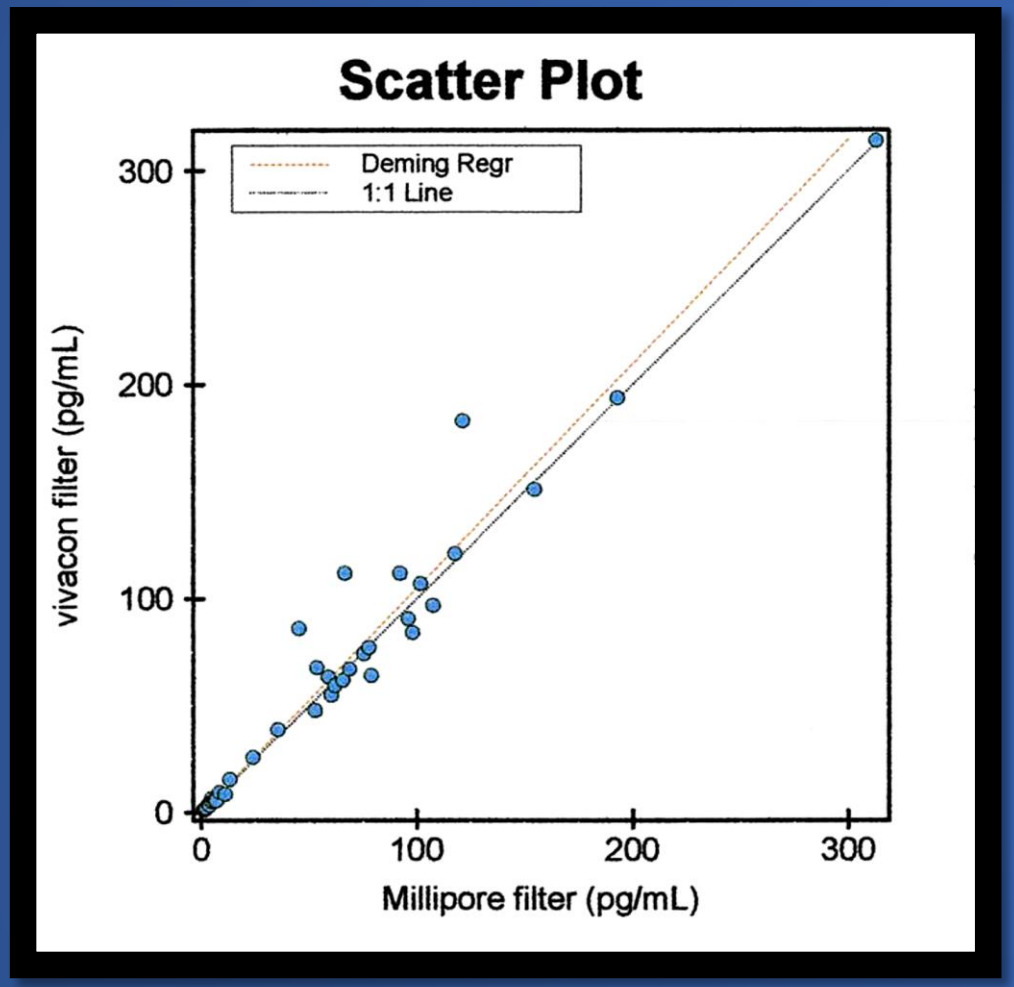
Bio-Rad controls assayed

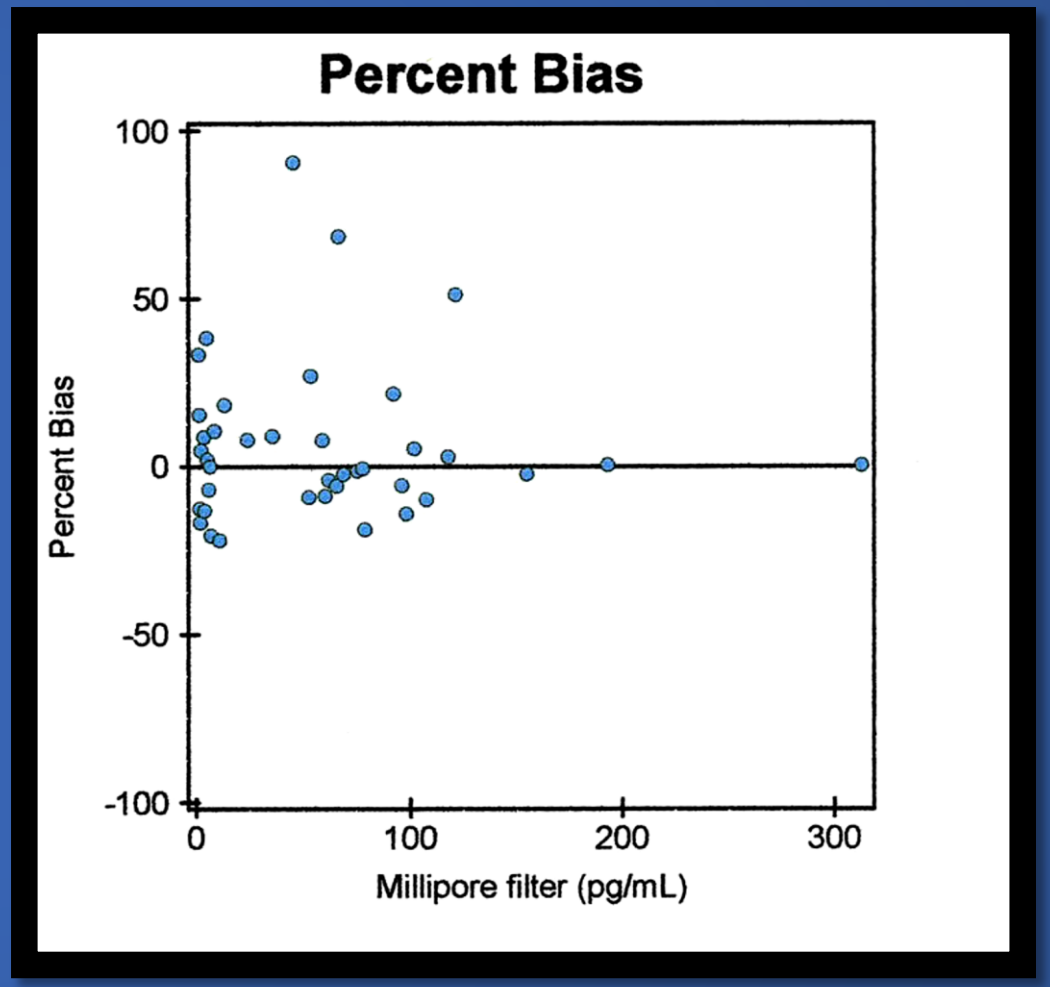
- High control
  - Samples tested (n) = 4
  - Mean 141.75 pg/mL
  - Observed CV = 5.8%
- Low Control
  - Samples tested (n) = 4
  - Mean 12.08 pg/mL
  - Observed CV = 3.0%

Conclusion

- High negative bias (-9.71%)
- Repeat comparison
  - Adjust spin time of Vivafree™ device to 20 min
  - 39 serum samples

Comparison Results - 20 min spin





Slope = 1.047
Corr Coef (R) = 0.9748

Average % Bias = 5.78

Intraday Precision -20 min spin

Bio-Rad controls assayed

- High control
  - Samples tested (n) = 10
  - Mean 188.09 pg/mL
  - Observed CV = 6.5%
- Low Control
  - Samples tested (n) = 10
  - Mean 16.02 pg/mL
  - Observed CV = 8.3%

Total Precision – 20 min spin

Bio-Rad controls assayed

- High control
  - Samples tested (n) = 52
  - Mean = 144.3 pg/mL
  - CV = 12.56%
- Low control
  - Samples tested (n) = 53
  - Mean = 13.0 pg/mL
  - CV = 10.18%

Conclusion

- The Vivafree™ device from Viva Products is comparable to the Millipore device for the determination of Free Testosterone
  - 20 min centrifugation time
  - Slope 1.047
  - r 0.9748
  - Precision ≤ 12.56%
- Substantial cost savings
  - 35% reduction
  - $42,000 annually
  - Better value
  - No additional tech time