

Evaluation of the Neobase Non-derivatized Kit

-The Missouri NBS Laboratory Experience-



Patrick Hopkins
Missouri NBS Laboratory Manager

Missouri MS/MS Profile

- ~90,000 screens received per year
- Using Neogram Derivatized kits
- Utilizing 2 Waters Quatro Micro systems
- Utilize full scan and some MRM scans
- 3 Scientists assigned to the testing area
- Have been expanded with MS/MS since 2005
- Have had two Malonic Aciduria cases.
- Have had no TYR type-I cases (TYR cutoff = 250 $\mu\text{mol/L}$).

Why did we consider Neobase?

- We liked the idea of getting away from the butanol-HCL component of sample processing.
- Sample processing would be easier to conduct and easier to cross-train other staff to do.
- It integrated the SUAC analyte to insure TYR type I detection (we could remove the caveat on our disorder list for TYR type I).
- We were told that the Neogram kits were destined to be phased out.

12-Week Evaluation Process

- Programmed the mass spec software to be able to switch back and forth from Neogram to Neobase methods (conducted parallels only on our slower days).
- Samples tested in parallel:
 - 2,302 Normals
 - 49 Various Confirmed Positives
 - 42 Borderline Abnormals
 - 130 Previous PT Samples

Issues Encountered with Neobase

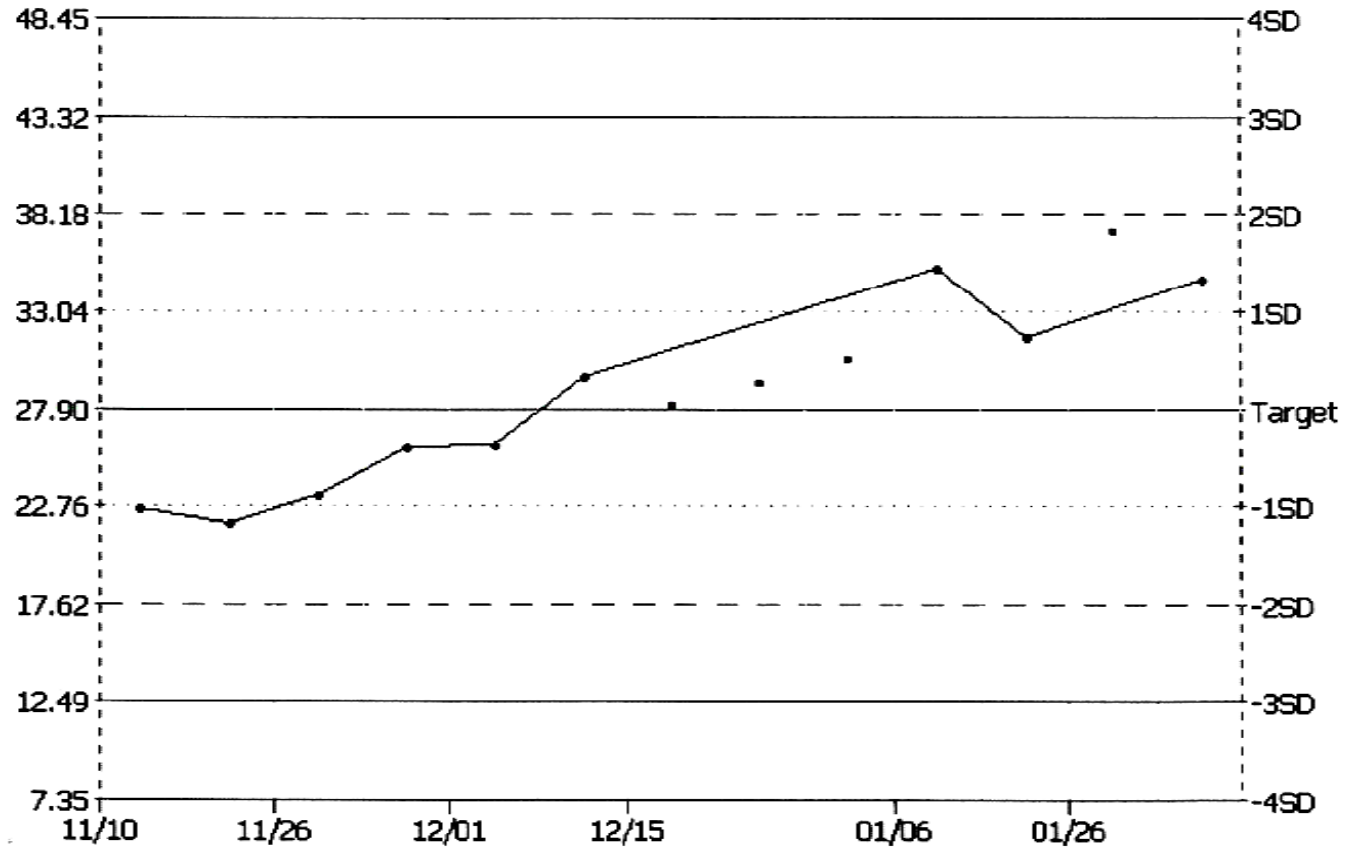
- Unable to keep the mass specs clean enough for an acceptable length of time (the amino acid low controls started dropping out of $-3SD$ after running 30 plates).
- Low recoveries for C3DC (14%) and SUAC (19%).
- Missed some PTs when testing former PT samples.
- Unable to identify a known Malonic Acidemia case as positive without lowering C3DC cutoff too near the mean.
- Instrument matching problems:
 - C0 = 20% dif
 - C5OH (C4DC) = 26% dif
 - C3DC (C4OH) = 37% dif
 - C16OH = 40% dif

Issues Encountered with Neobase

- Must run all MRM mode; No full scan available without running an extra sample.
- Unable to detect FIGLU.
- Pipetting for sample prep more prone to human error – requires 2 pipette settings to remove volume of extraction solution and to add amino acids and acylcarnitines.
- The Free Carnitine (CO) mean drifted upwards considerably as the instruments got dirty.

Free Carnitine Means Drift with Neobase

CO - Mean of Initials



Weighing Pros and Cons

- Met with my MS/MS section manager and senior scientist to get everyone's input and honest opinions.
- We sat down and went through all the pros and cons and asked ourselves if we would be seeing a “net improvement” in our MS/MS testing area.
- The answer kept coming up as “No”. We were more pleased with our current method and the results and QA/QC that we are currently maintaining.
- The issue of the dirtier mass specs and the problems that this caused seemed to be the final deal stopper for us.

Final Outcomes

- Decided to stick with the Neogram Derivatized method with the hopes that some of these issues with the Neobase could somehow be worked out down the road by the vendor.
- We reported our findings to our Genetics Advisory Committee and they concurred with our decision to not switch to this method at this time.
- Our building maintenance staff know the issues with the slow corrosion to the fume hood vents and they keep a close eye on it.