Case Study: Driving Specialized Testing through Assay Development

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bioMONTR

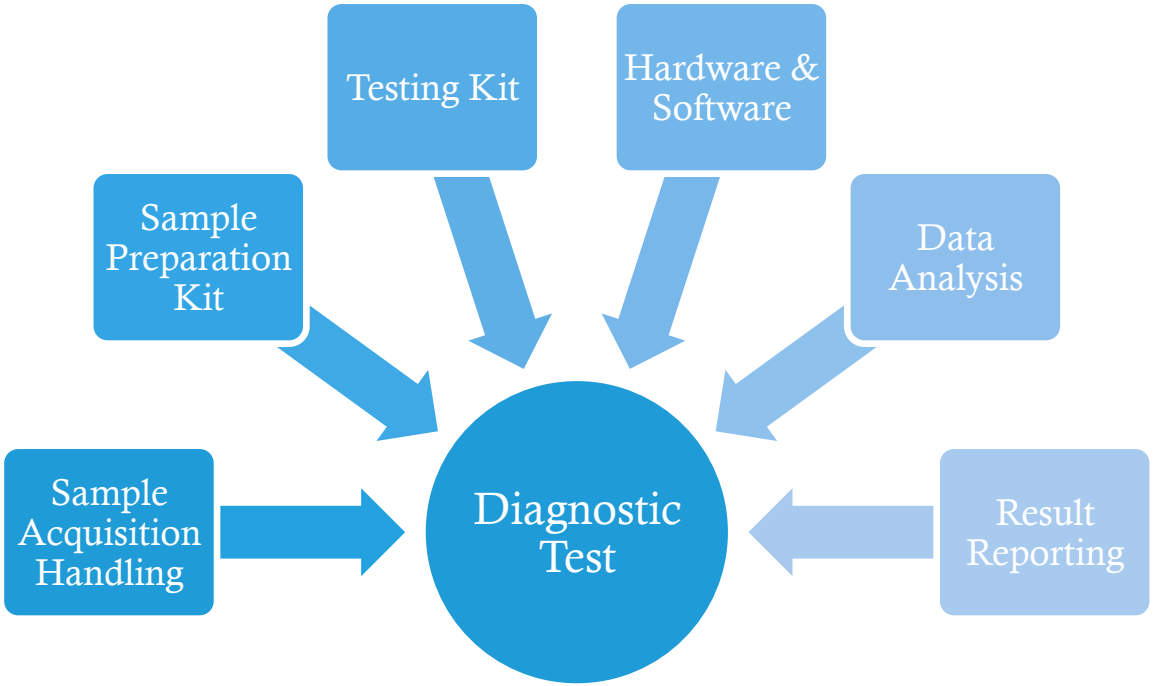
Disclosures

- McClernon LLC and bioMONTR provide consulting and assay development services for the following:
  - Abbott Molecular
  - Celera
  - DNA Genotek, Inc.
  - GlaxoSmithKline LLC
  - Quest Diagnostics
  - ViiV Healthcare
  - Vivebio LLC

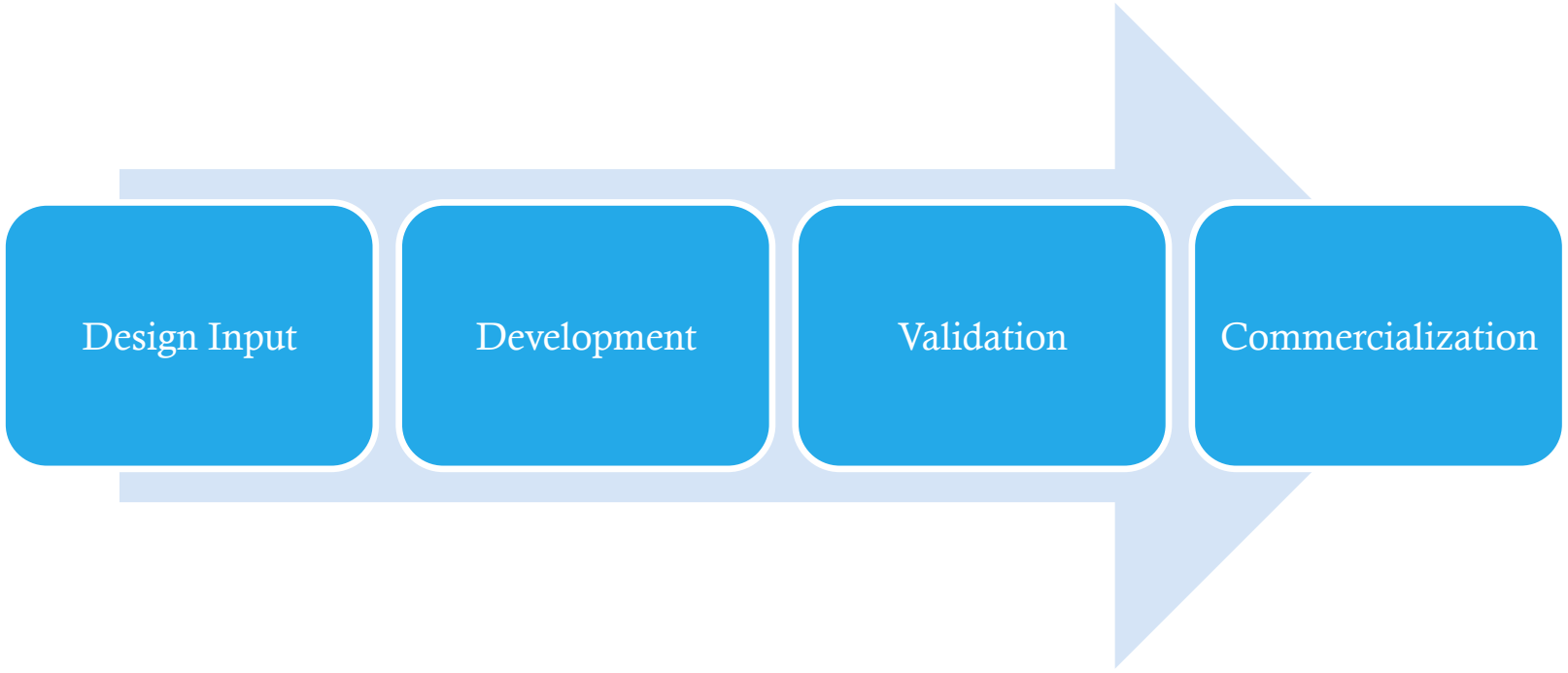
Objectives

- Trace development of a molecular diagnostic assay for an unmet clinical need
- Describe potential benefits of collaborative relationships with pharma and external researchers in bringing up novel tests
- Discuss how assay development is imperative to drive specialized testing

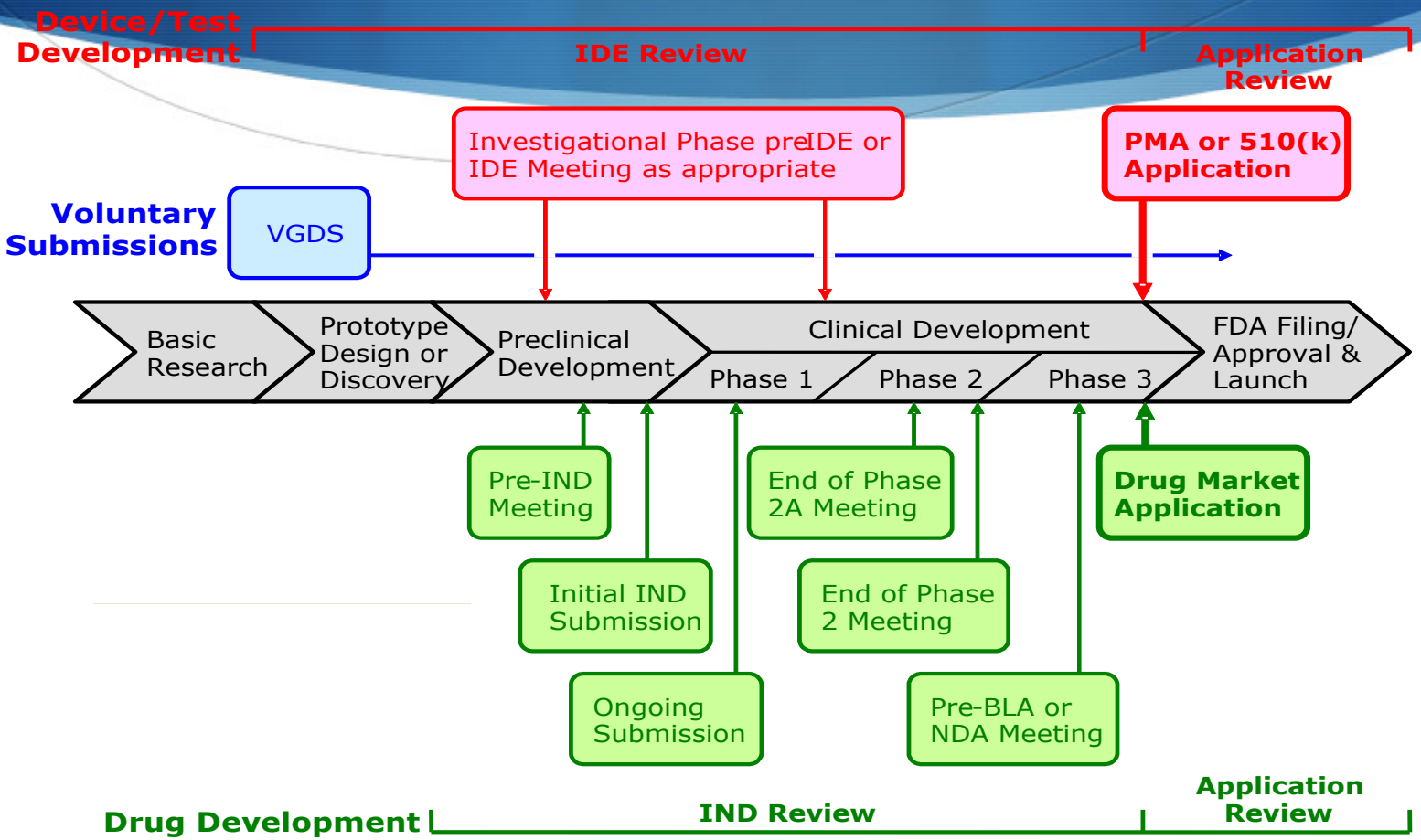
Entire System ≠ Individual Pieces of a Test



Typical Assay Development Process



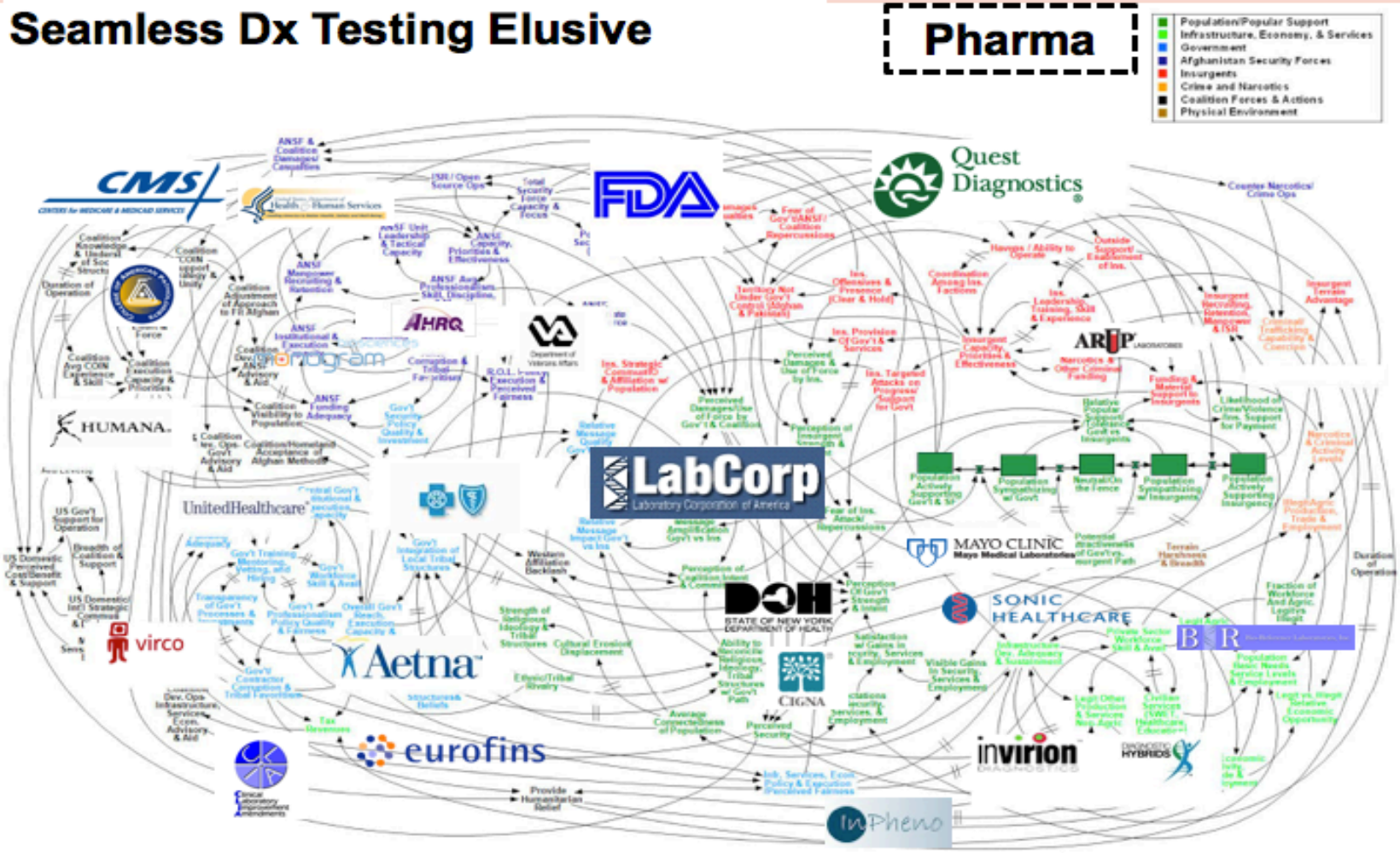
Drug-Device Co-Development Process



Drug-Diagnostic Co-Development Concept Paper – DRAFT (FDA, April 2005)

Seamless Dx Testing Elusive

Pharma



HIV-1 Viral Load

- Used to determine efficacy of highly active antiretroviral therapy (HAART)
- Suppression to <50 c/mL is standard of care……treatment regime is working
- However, 10-15% of HIV infected individuals on therapy and fully suppressed (<50 c/mL) go on to experience neurological cognitive dysfunction
- What biomarkers could be used to determine/predict which cohort of individuals will have issues
- Could detection of HIV-1 RNA below the current <50 c/ml cutoff be used as a sufrogate biomarker for drug penetration across the blood:brain barrier in HIV+ individuals?

Summary of Assays for HIV Viral Load

Company	Assay Name	Technique	Sample Volume (mL)	Range (c/mL)
Abbott Molecular	RealTime HIV-1 Viral Load	RT-PCR	0.6 – 1.2	40-10M
Siemens Healthcare	Versant HIV-1 3.0 (bDNA)	Hybridization/ signal amplification	1	50/75-500,000
bioMerieux	NucliSENS EasyQ v1.2	NASBA		
Roche	Amplicor HIV-1 Monitor v1.5	RT-PCR	0.5	50-750,000
Roche	COBAS Ampliprep/ COBAS TaqMan	RT-PCR	0.5	48-10M
Qiagen	QIAsymphony	RT-PCR		

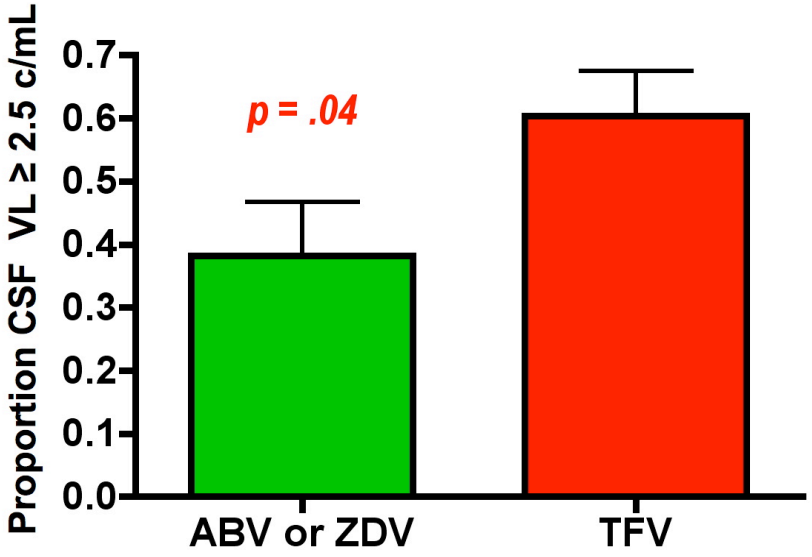
CHARTER Study Design

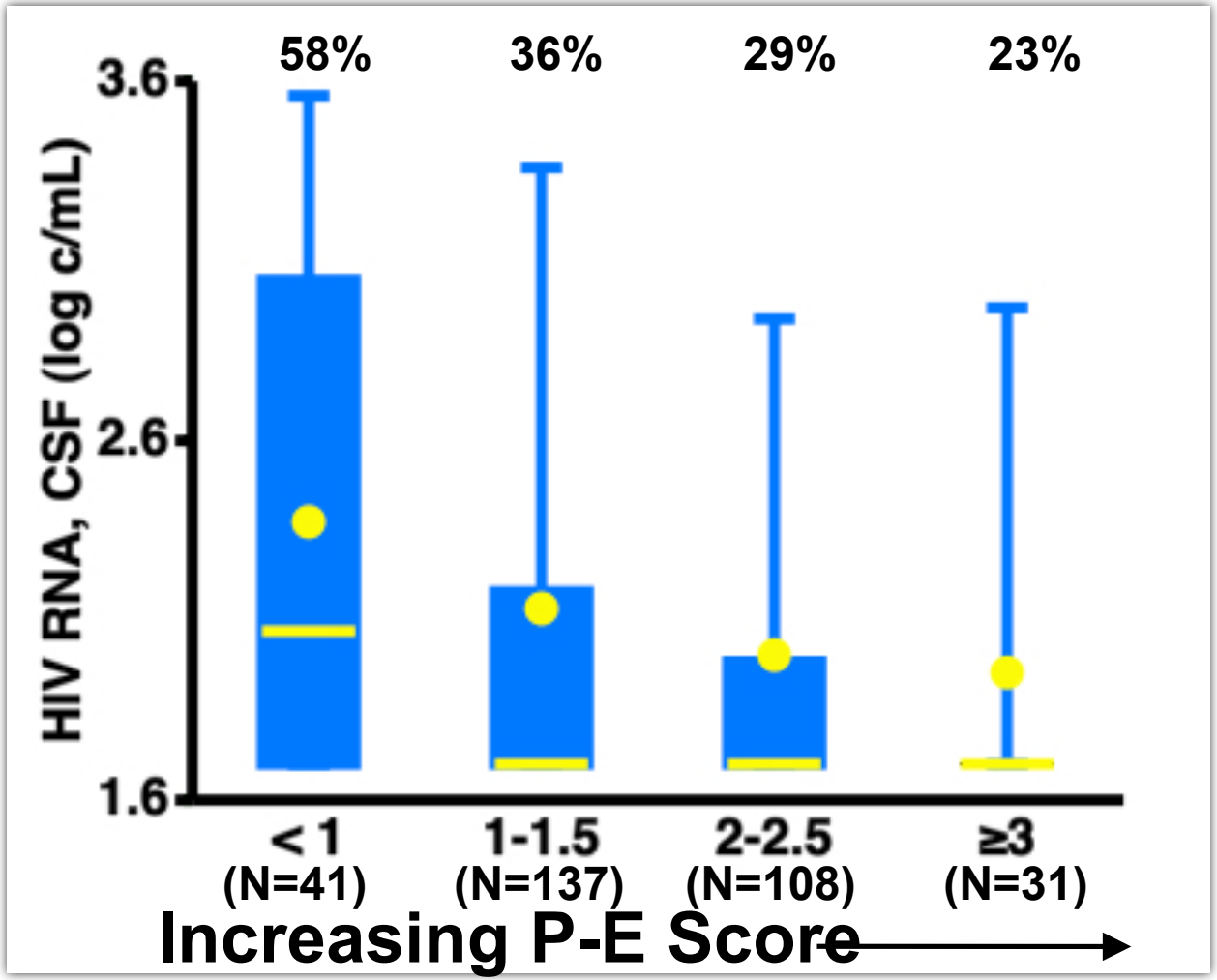
- Participants
  - 317 HIV-infected individuals from the CHARTER cohort who completed standardized assessments, had successful lumbar punctures, and had HIV RNA levels measured in both plasma and CSF (<50 c/mL with FDA approved assay)
- Laboratory Procedure
  - Utilized a SuperLow HIV-1 assay (modified protocol) and experimental algorithm that allows detection of HIV-1 to 2 copies/mL RNA (EasyQ commercially available from bioMerieux)

CHARTER Study Objectives

- Determine the proportion of CSF specimens that had RNA >2 c/mL
- Determine the correlates of HIV RNA levels >2 c/mL, including the effect of antiretroviral penetration

- Lower CPE Scores trended towards being associated with detectable HIV in CSF (p = 0.09)
- TFV users had more than twice the odds of having detectable HIV in CSF as users of either ABV or ZDV (OR 2.46, p = 0.04)




58%
36%
29%
23%
3.6
2.6
1.6
HIV RNA, CSF (log c/mL)
< 1
(N=41)
1-1.5
(N=137)
2-2.5
(N=108)
≥3
(N=31)
Increasing P-E Score

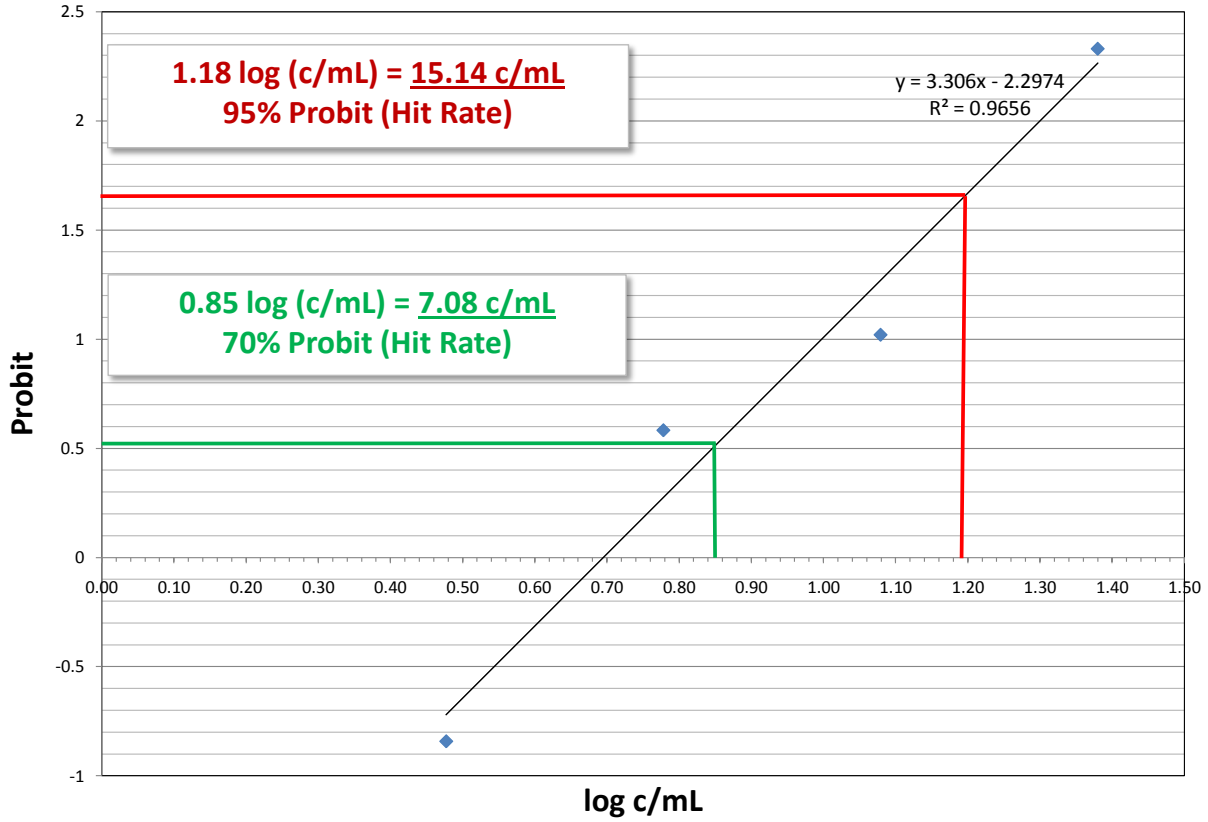
Study Conclusions

- Reduced antiretroviral penetration was associated with detectable HIV in CSF based on our HIV-1 SuperLow assay
- SuperLow HIV-1 real time assay detected HIV RNA in 49% of CSF specimens that were undetectable by the standard Roche COBAS assay. We conclude a more sensitive HIV viral load assay may be needed to monitor CSF neurological impaired individuals.
- Demonstrated possible clinical utility of SuperLow HIV-1 VL assay in HIV CNS disease. Prospective trials have been initiated with collaborative groups funded by HIV pharma companies.

HIV-1 SuperLow Assay: Precision

	Target Input (copies/mL)						
	3	**6**	**12**	**24**	**48**	**72**	**96**
N	25	25	26	35	27	27	27
Mean, c/mL	3	6	9	23	52	83	75
Std Dev, c/mL	1	6	7	18	41	56	41
Mean, LOG c/mL	0.33	0.61	0.81	1.23	1.60	1.83	1.81
Std Dev, LOG c/mL	0.28	0.36	0.32	0.34	0.33	0.26	0.25

HIV-1 SuperLow: Probit Analysis



HIV-1 SuperLow Assay: Performance Characteristics

- Proprietary bioMONTR Assay
- RESEARCH USE ONLY
- Performance Characteristics
  - Plasma, Serum or CSF (up to 2 mL)
  - Limit of Detection: 2 c/mL
  - Reportable Range: 2 to 10 M c/mL
  - 70% hit rate: 7 c/mL; 95% hit rate: 15 c/mL (with 2 mL sample input)
- Clinical Specimen Study: Retrospective analysis of 251 plasma specimens previously <50 c/mL with FDA approved assay. 37% (n = 92) yielded results ranging from 3 – 400 c/mL with SuperLow Assay. 63% (n = 158) were <2 c/mL with SuperLow Assay.

Timeline: CSF HIV-1 RNA as a possible biomarker for HIV drug efficacy and HIV patient management

1995-2002	• Researchers determine HIV-1 RNA in CSF could be a used as a biomarker/ predictor for HIV associated neurological dysfunction.
2002-2009	• Assay development in association with HIV drug development programs work to establish correlation/clinical utility of monitoring low HIV-1 viremia in HIV+ population.
2009 - Ongoing	• Prospective clinical trials initiated to determine clinical utility of HIV-1 low viremia test in combination with new HIV drug development = change in clinical management of HIV+ cohort experiencing neurological dysfunction

Collaborative Efforts

Diagnostics

Pharmaceuticals

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THANK YOU

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