Standardization of Laboratory Assays for HCV Drug Resistance: Opportunities and Challenges

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HCV DRAG

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CFAR
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- Laboratory issues
- Issues in discovery, clinical development and clinical practice

A Final Lesson from HIV: Standardization is not without Pitfalls

- Assays initially grow up in different settings but may not always capture the entire picture

Recombinant Soluble CD4

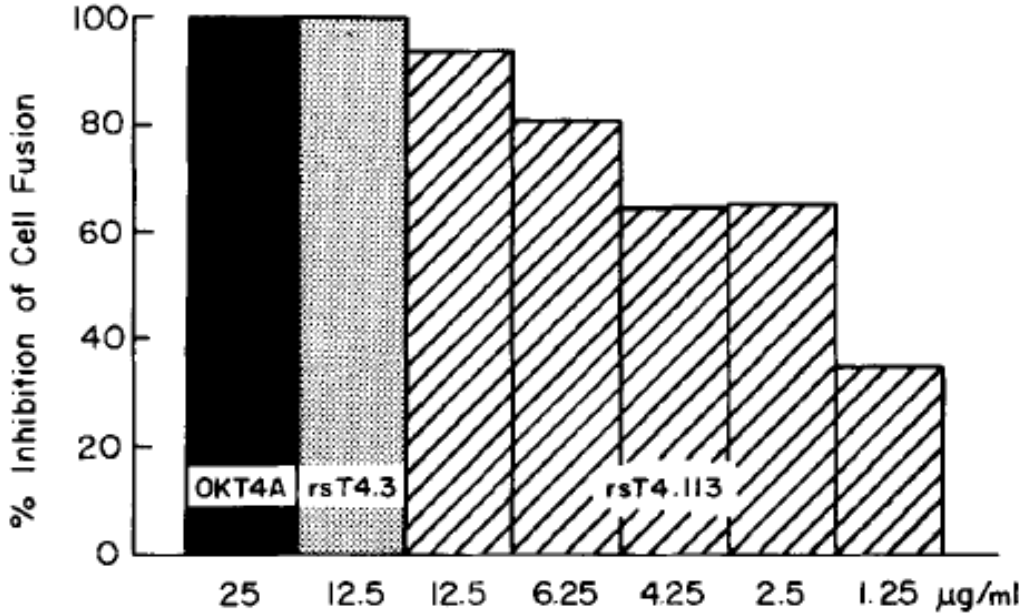


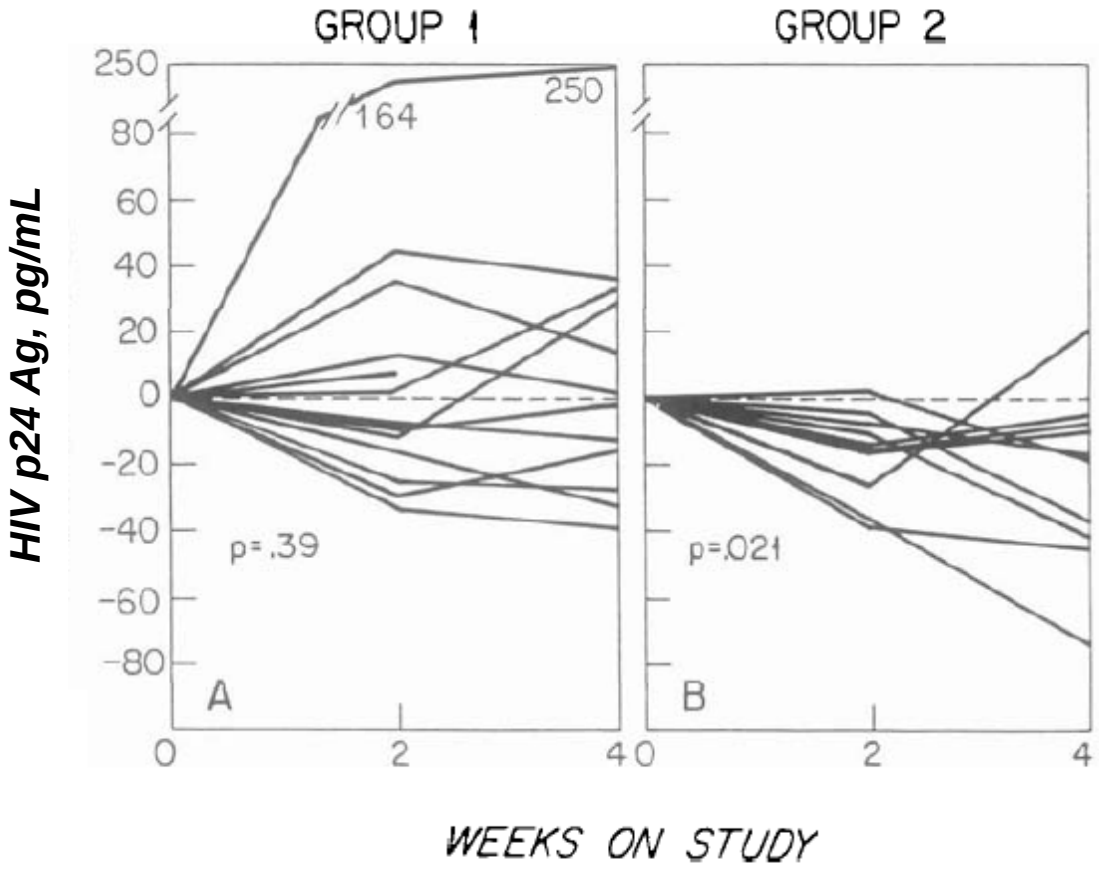
FIG. 5. **Antiviral activity of rsT4.113.** rsT4.113 exhibits a dose-dependent activity in neutralization of HIV-induced cell fusion between a $T4^+$ C8166 cell line and a chronically infected H9 cell line. Percent inhibition is calculated based upon control values for number of syncytia in assays lacking rsT4 or OKT4a.

Chao, et. al, J Biol Chem 1989

Great Idea; Bad Result

- 40 patients received up to 30 mg/day of rsCD4
- Measured serum concentrations of rsCD4 high enough to have predicted a much more significant effect

HIV-1 p24 Ag Response to rsCD4 *in vivo*

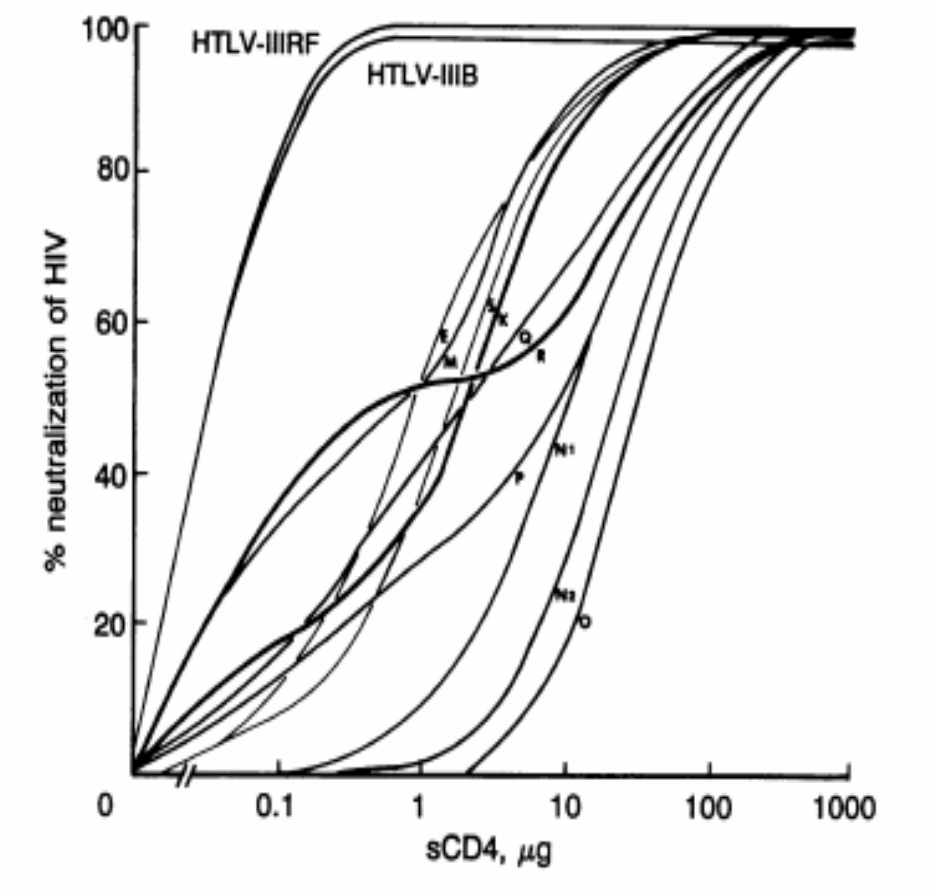


Schooley, et.al, Annals of Internal Medicine, 1990

Great Idea; Bad Result

- 40 patients received up to 30 mg/day of rsCD4
- Measured serum concentrations of rsCD4 high enough to have predicted a much more significant effect
- Trend in reduction of circulating HIV-1 p24 antigen in patients receiving highest dose of rsCD4

Reduced Susceptibility of Clinical Isolates of HIV-1 to sCD4



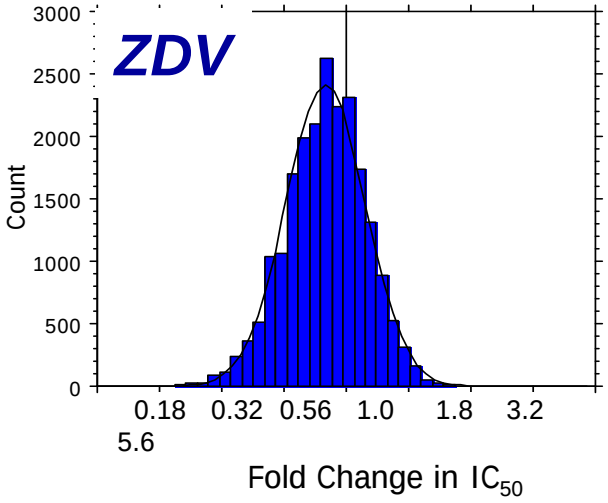
Daar, et. al. PNAS, 1990

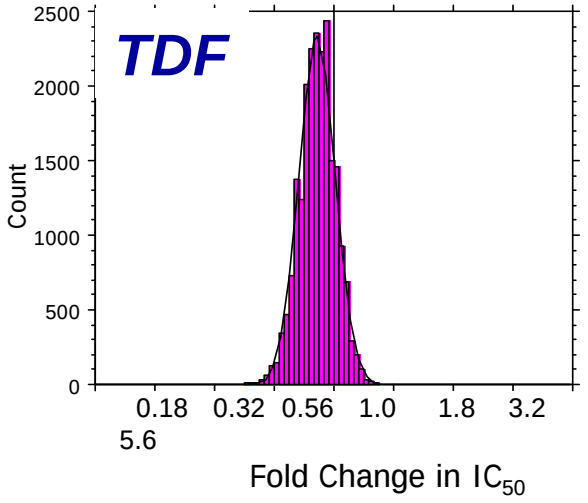
Sensitization of HIV-1 to rsCD4 by Laboratory Passage

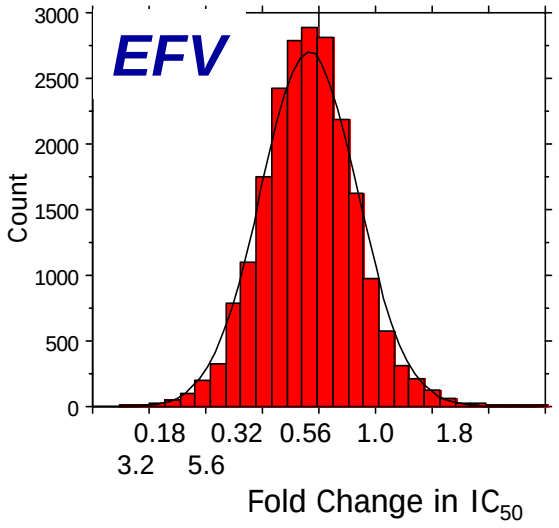
Virus	Passage day	rsCD4 blocking conc., μg/ml			
		1 TCID	5 TCID	25 TCID	125 TCID
HIV-1/IIIB	>100	<1	<1	<1	<1
C-08	14	ND	10–100	10–100	100
	44	1	1	3	10
	67	0.3	0.3	1	ND
C-17	14	100	100	100	300
	44	3	3	10	30
	67	0.3	1	3	ND

Turner, et. al., PNAS, 1992

WT Susceptibility in HIV can be Broad Depending on the Agent

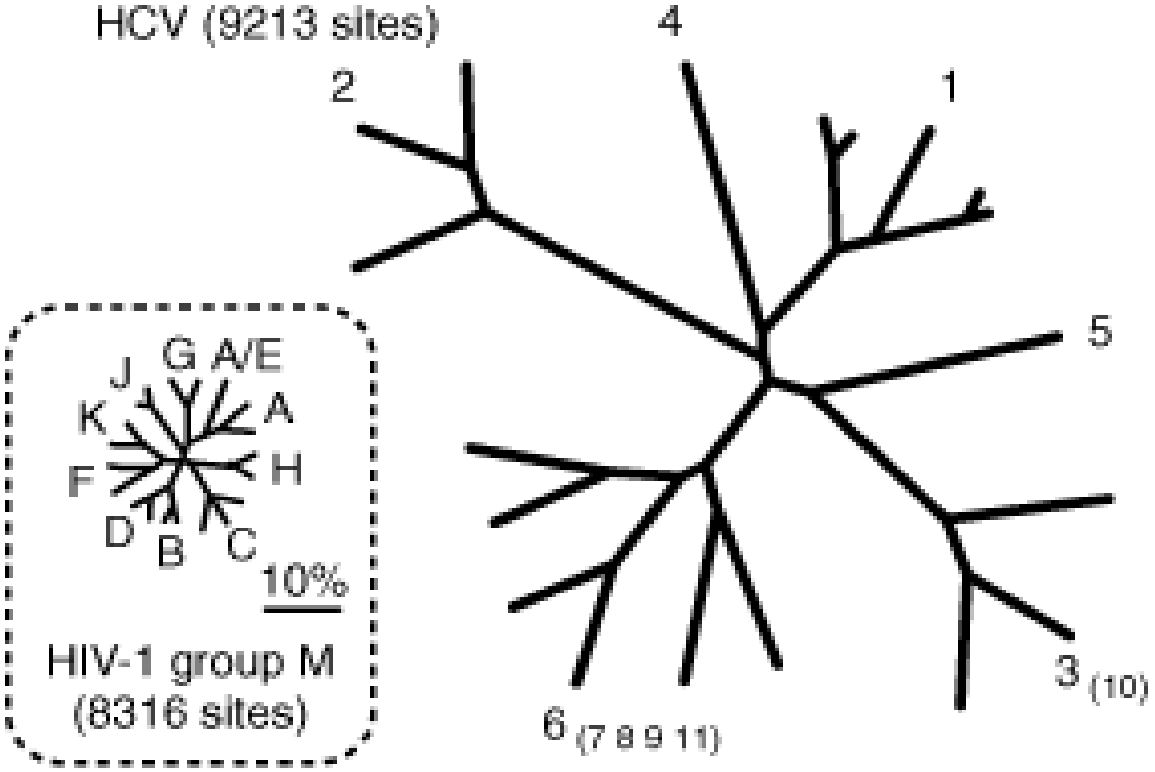






Monogram Biosciences

Genetic Diversity of HCV makes HIV Look "Clonal"



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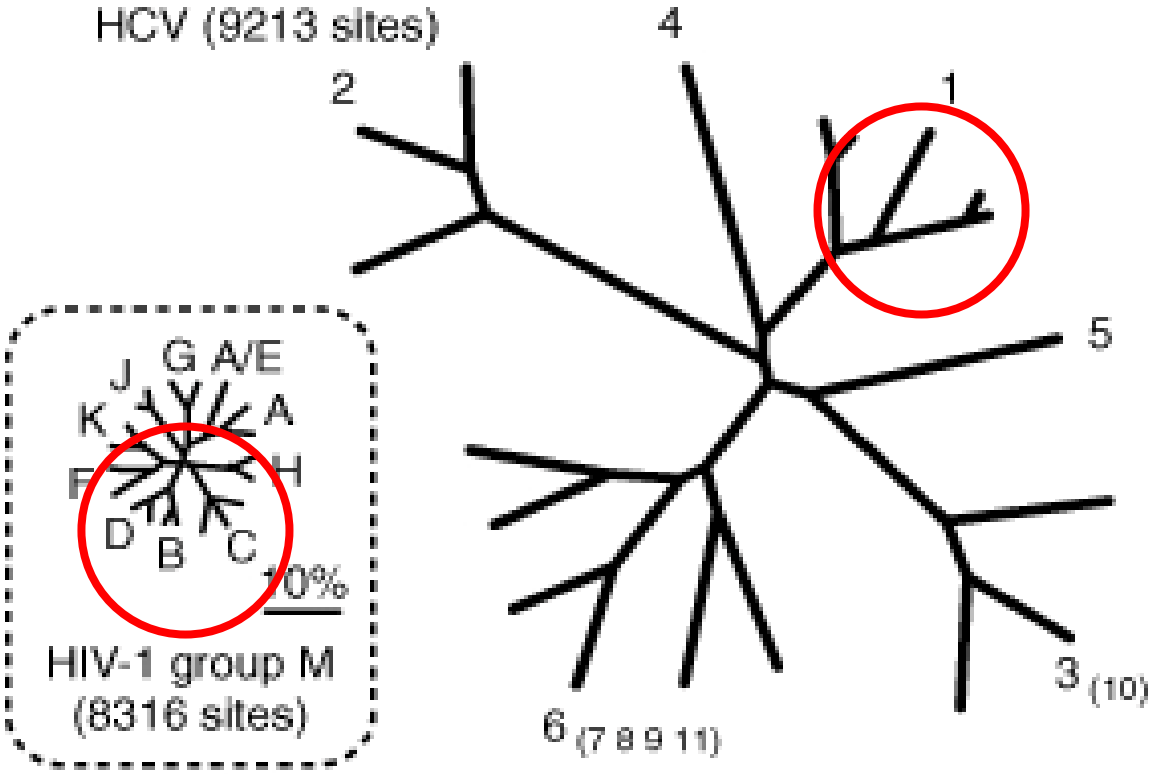

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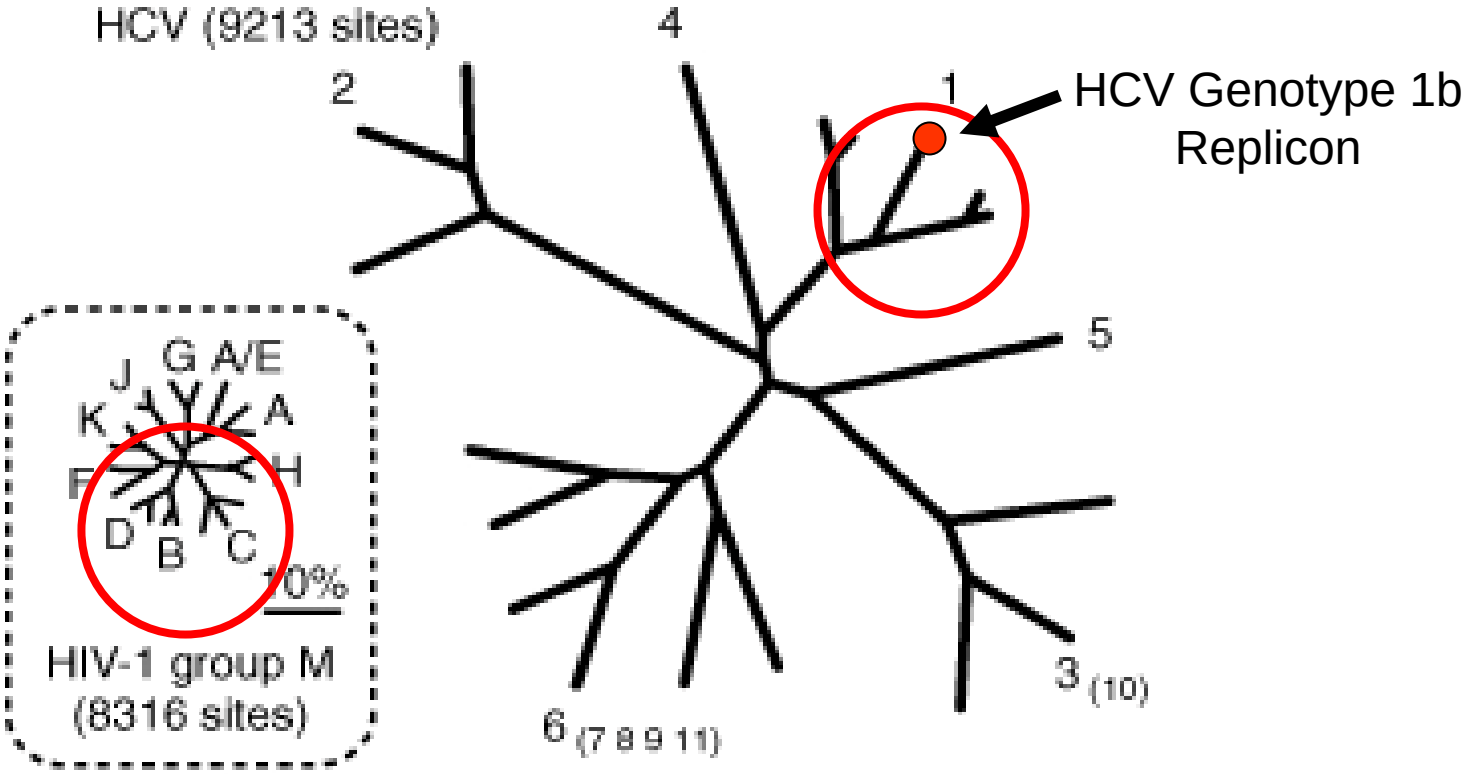
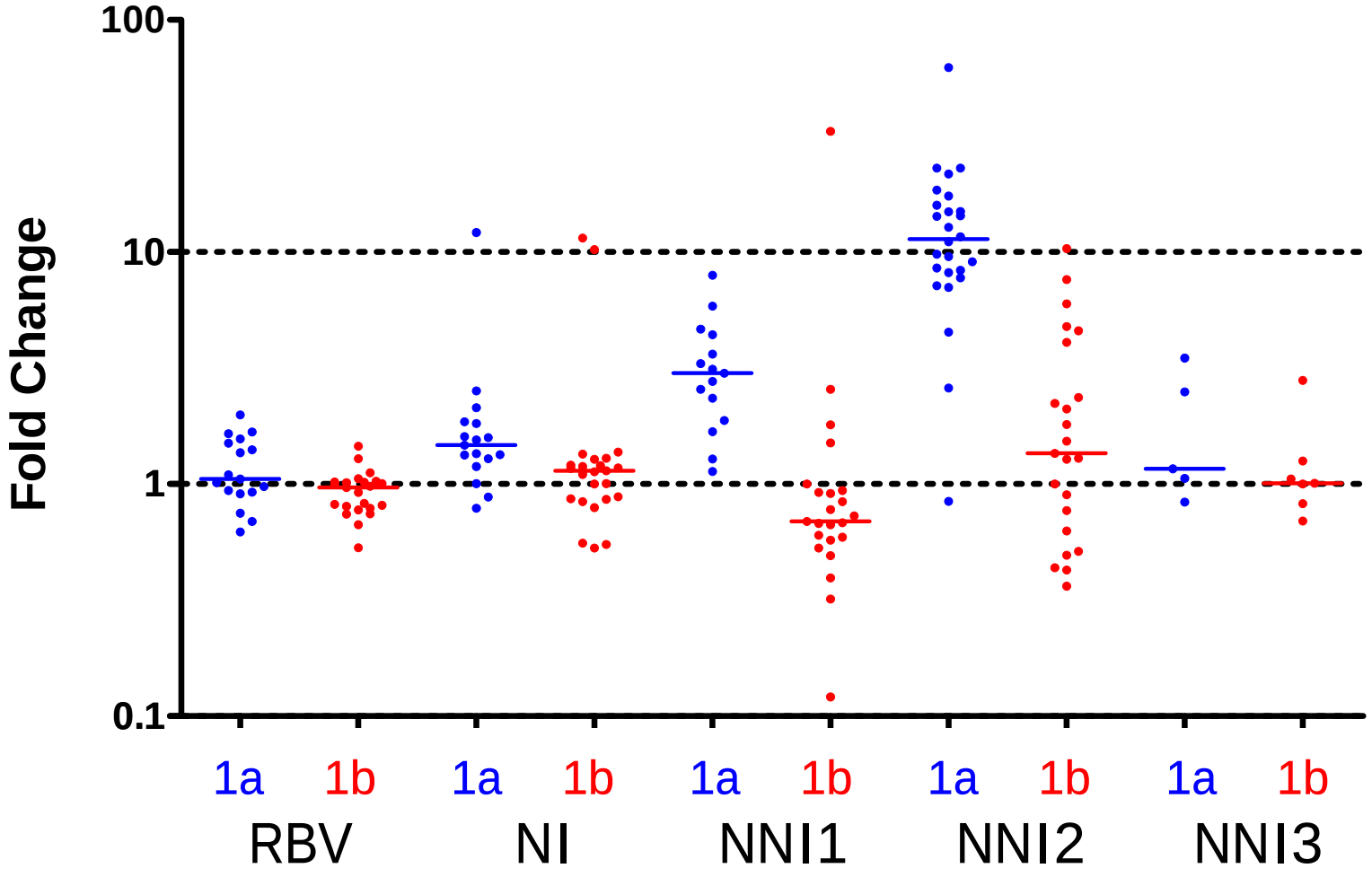


Patient Sample Drug Susceptibility Split by Subtype (1a/1b)



Courtesy of Neil Parkin, Ph.D. Monogram Biosciences

The Cottage Industry of HIV Laboratory Studies

- A number of assays for viral quantification were developed in academic and pharmaceutical laboratories and used in clinical trials.
- One of the first clinical trials to use a viral endpoint was the ACTG rsCD4 trial that used HIV-1 p24 Ag
- It soon became apparent as had been the case with CD4 cell testing that standardization was needed.

HIV Quantification QA Programs

Standardization of Sensitive Human Immunodeficiency Virus Coculture Procedures and Establishment of a Multicenter Quality Assurance Program for the AIDS Clinical Trials Group

F. BLAINE HOLLINGER,[1,2*] JAMES W. BREMER,[1] LAWRENCE E. MYERS,[3] JONATHAN W. M. GOLD,[4] LISA McQUAY,[3] AND THE NIH/NIAID/DAIDS/ACTG VIROLOGY LABORATORIES†

Establishment of a Quality Assurance Program for Human Immunodeficiency Virus Type 1 DNA Polymerase Chain Reaction Assays by the AIDS Clinical Trials Group

J. BROOKS JACKSON,[1*] JAMES DREW,[2] HSIANG JU LIN,[3] PATRICIA OTTO,[2] JAMES W. BREMER,[3] F. BLAINE HOLLINGER,[3,4] STEVE M. WOLINSKY,[2] THE ACTG PCR WORKING GROUP,† AND THE ACTG PCR VIROLOGY LABORATORIES‡

Evaluation of a Quality Assurance Program for Quantitation of Human Immunodeficiency Virus Type 1 RNA in Plasma by the AIDS Clinical Trials Group Virology Laboratories

BELINDA YEN-LIEBERMAN,[1] DONALD BRAMBILLA,[2] BROOKS JACKSON,[1†], JAMES BREMER,[3] ROBERT COOMBS,[4] MIKE CRONIN,[5] STEVEN HERMAN,[6] DAVID KATZENSTEIN,[7] SHIELA LEUNG,[2] HSIANG JU LIN,[8] PAUL PALUMBO,[9] SURAIYA RASHEED,[10] JOHN TODD,[11] MARYANNE VAHEY,[12] AND PATRICIA REICHELDERFER[13,4]

J Clin Micro 1992, 1993 and 1996

Proficiency Testing for Genotypic Assays for HIV Drug Resistance

Model for Assessment of Proficiency of Human Immunodeficiency Virus Type 1 Sequencing-Based Genotypic Antiretroviral Assays

Diana D. Huang,[1]* James W. Bremer,[1] Donald J. Brambilla,[2] and Paul E. Palumbo,[3]
for the Pediatric ACTG Sequencing Working Group

Quality Control Trial for Human Immunodeficiency Virus Type 1 Drug Resistance Testing Using Clinical Samples Reveals Problems with Detecting Minority Species and Interpretation of Test Results

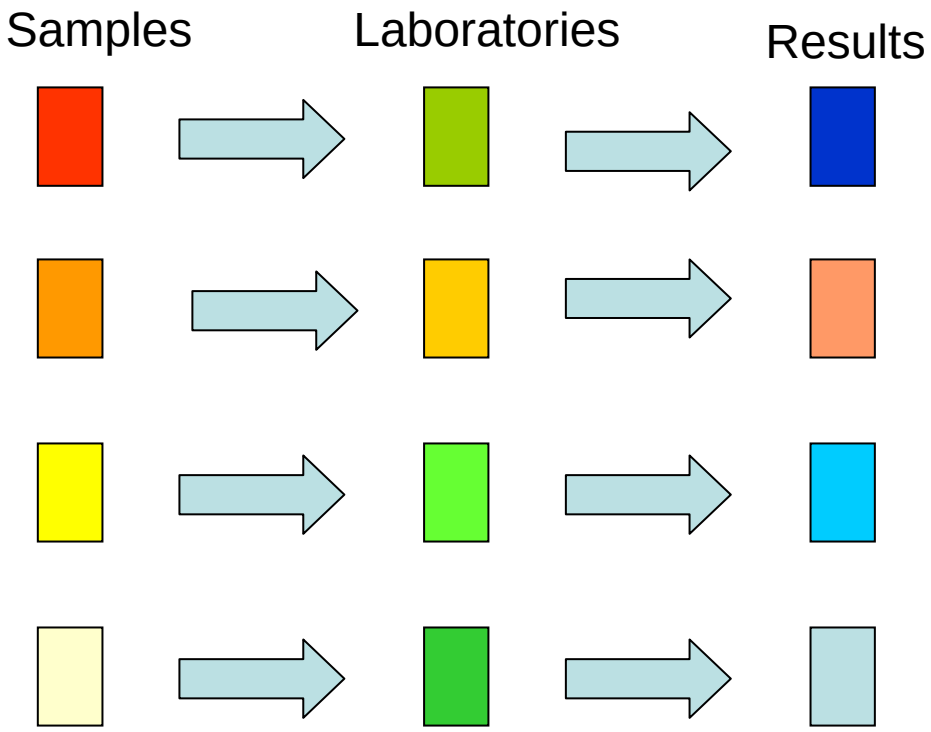
Klaus Korn, Heide Reil, Hauke Walter, and Barbara Schmidt*

J Clin Micro 2003 and 2005

Standardization Efforts: Common Features in Evolution of Programs

- Recognition by individual investigators that a number of variables can affect the results of an assay
- Recognition of seemingly discrepant results from different laboratories studying the “same thing”
- Realization by stake holders that assay standardization is required to minimize confusion by all involved

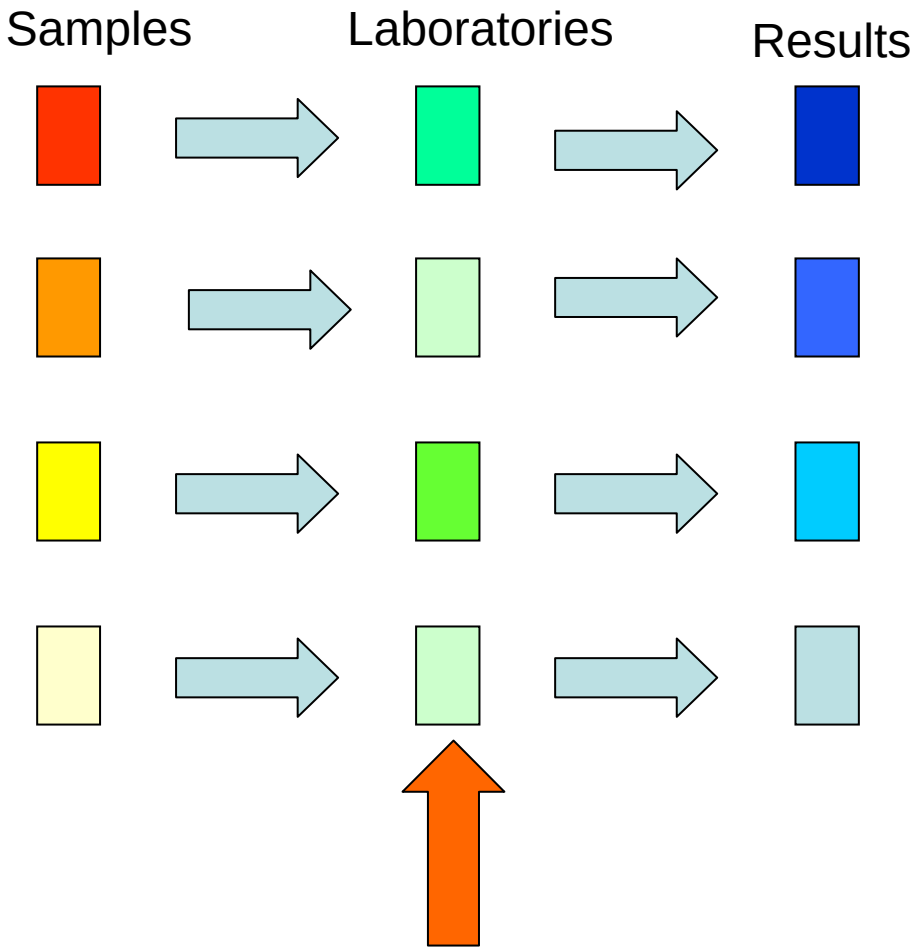
HIV Quantification and Genotypic Resistance Testing: Standardization in Multiple Laboratories



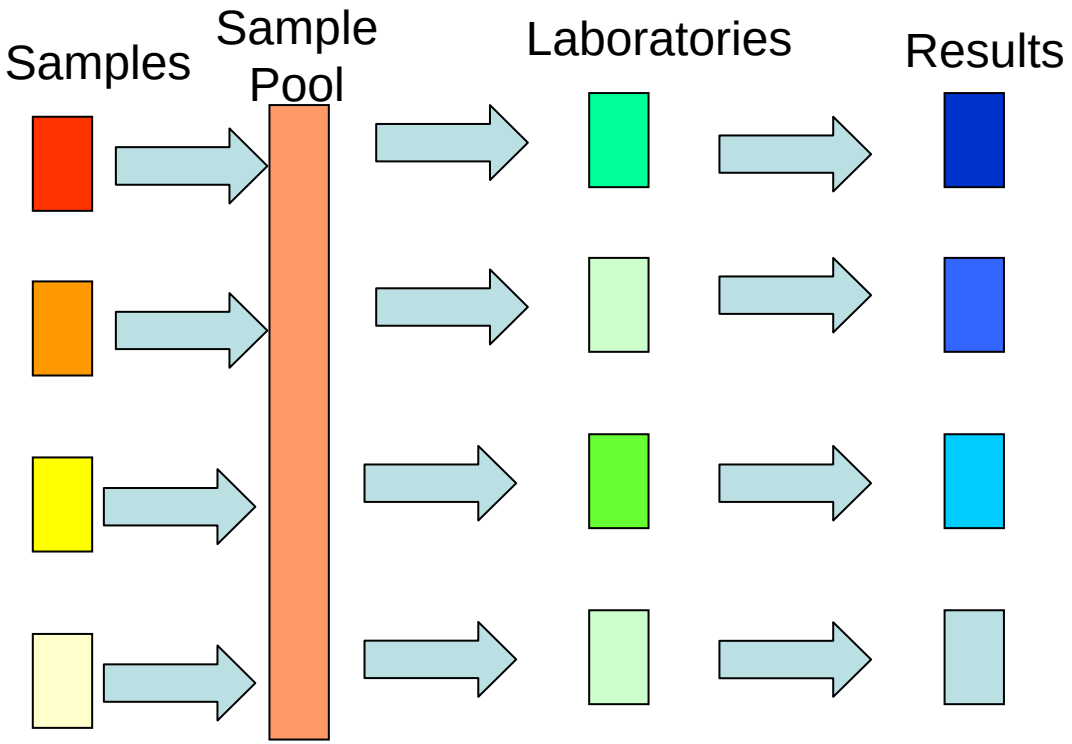
Standardization Efforts: Examples of Common Variables

- Viral strains
- Media and buffers
- Culture conditions
- Multiplicity of infection
- Time in culture
- Probes and primers
- Sample acquisition, processing, storage and shipping

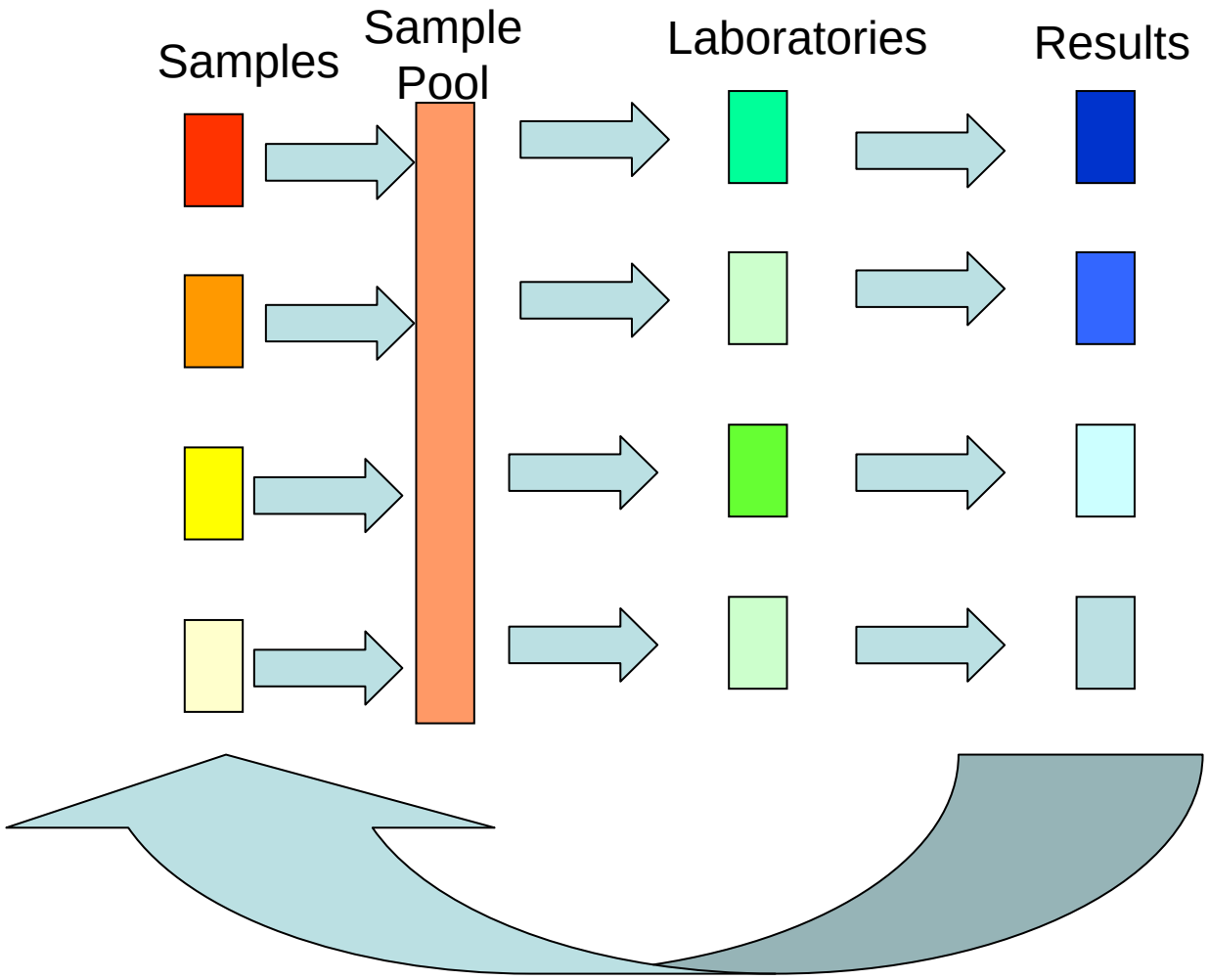
Step 1: Common Protocols and Reagents



Step 2: Create Pools of Known and/or Unknown Samples



Step 3: Iterative Refinement of Approach Through Repetitive Assay Cycles



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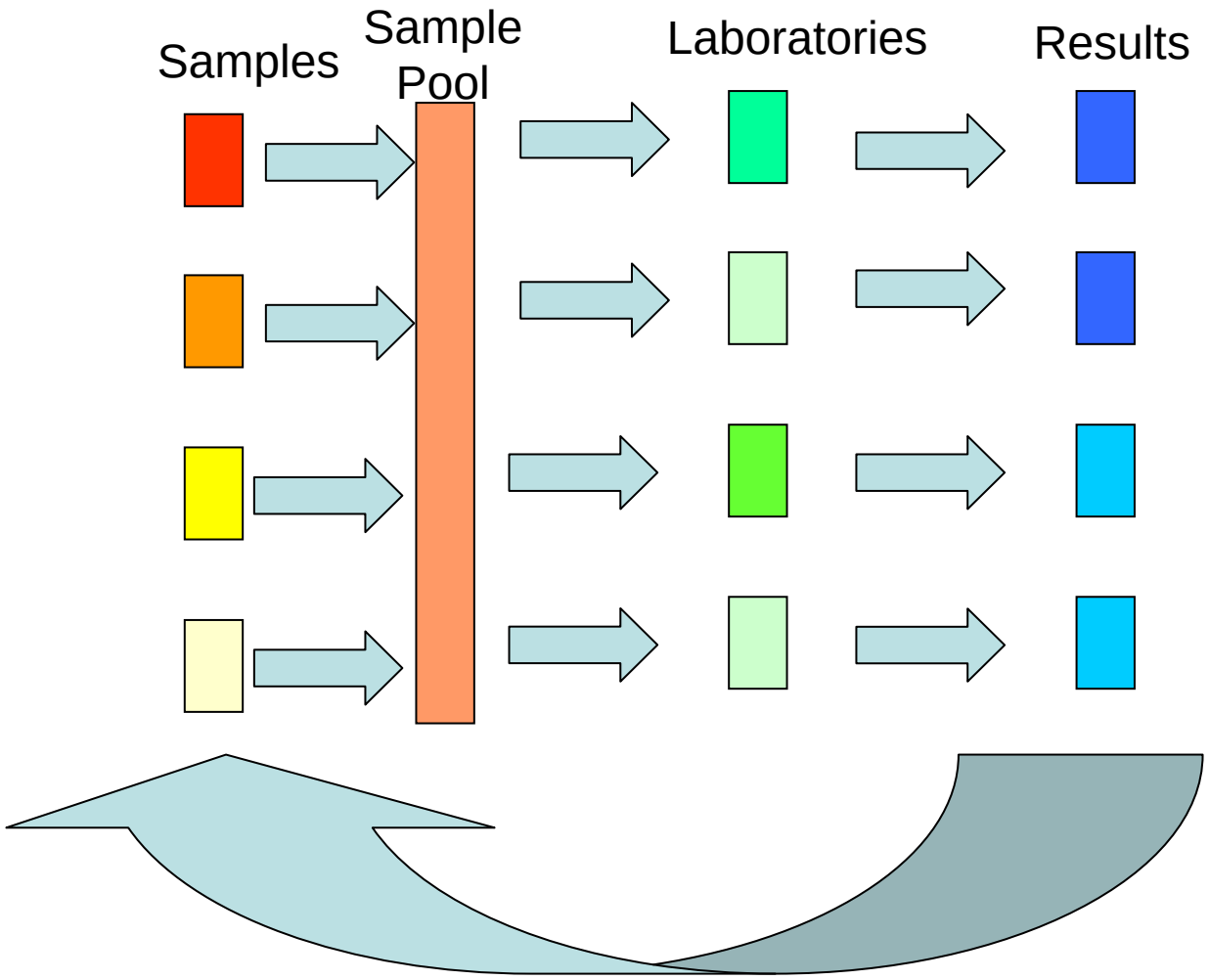
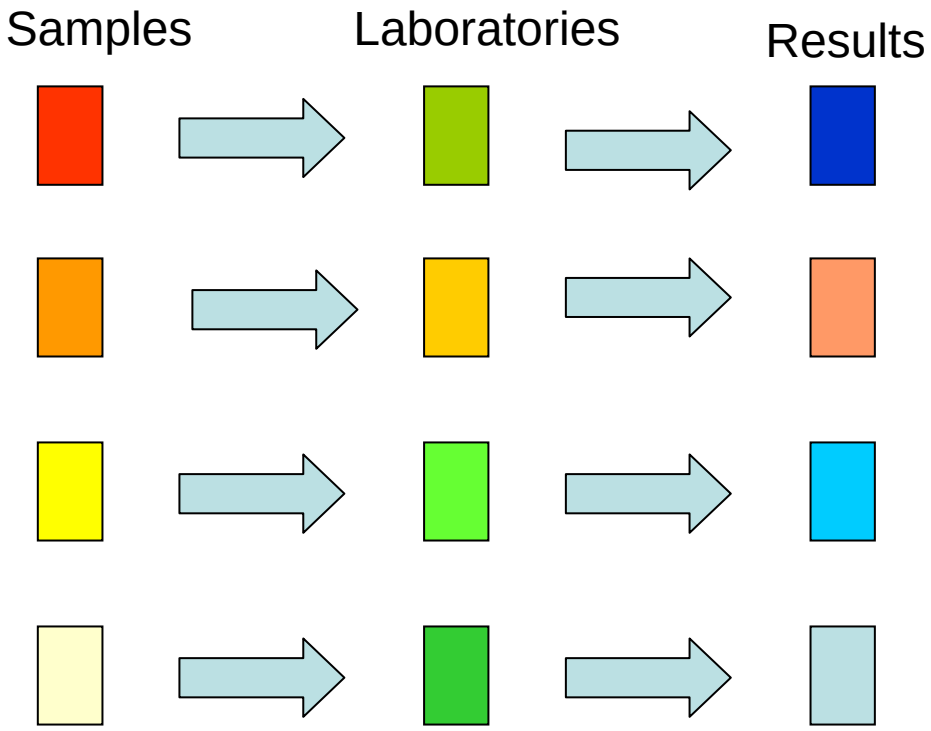


HIV Phenotypic Resistance Testing



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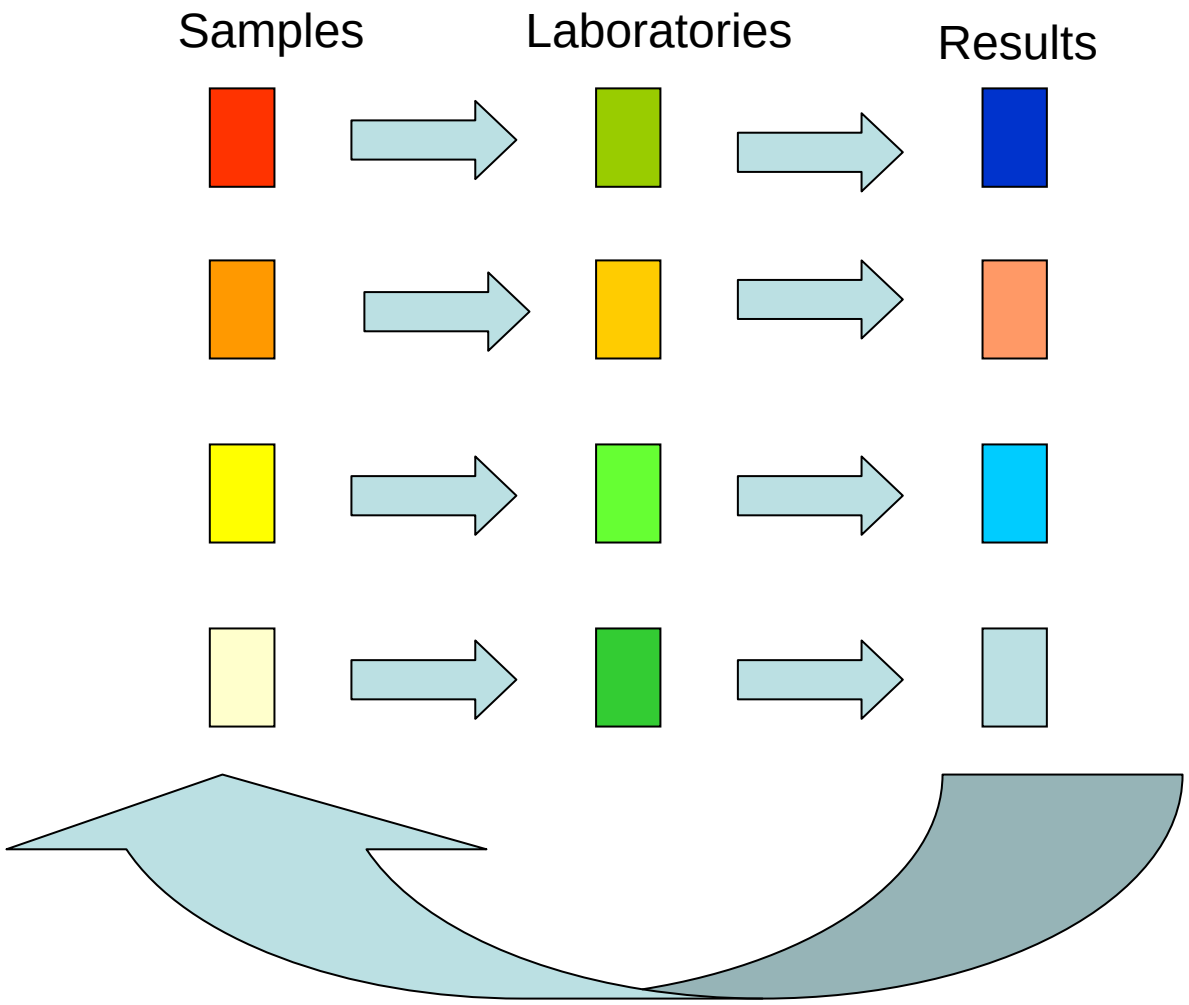
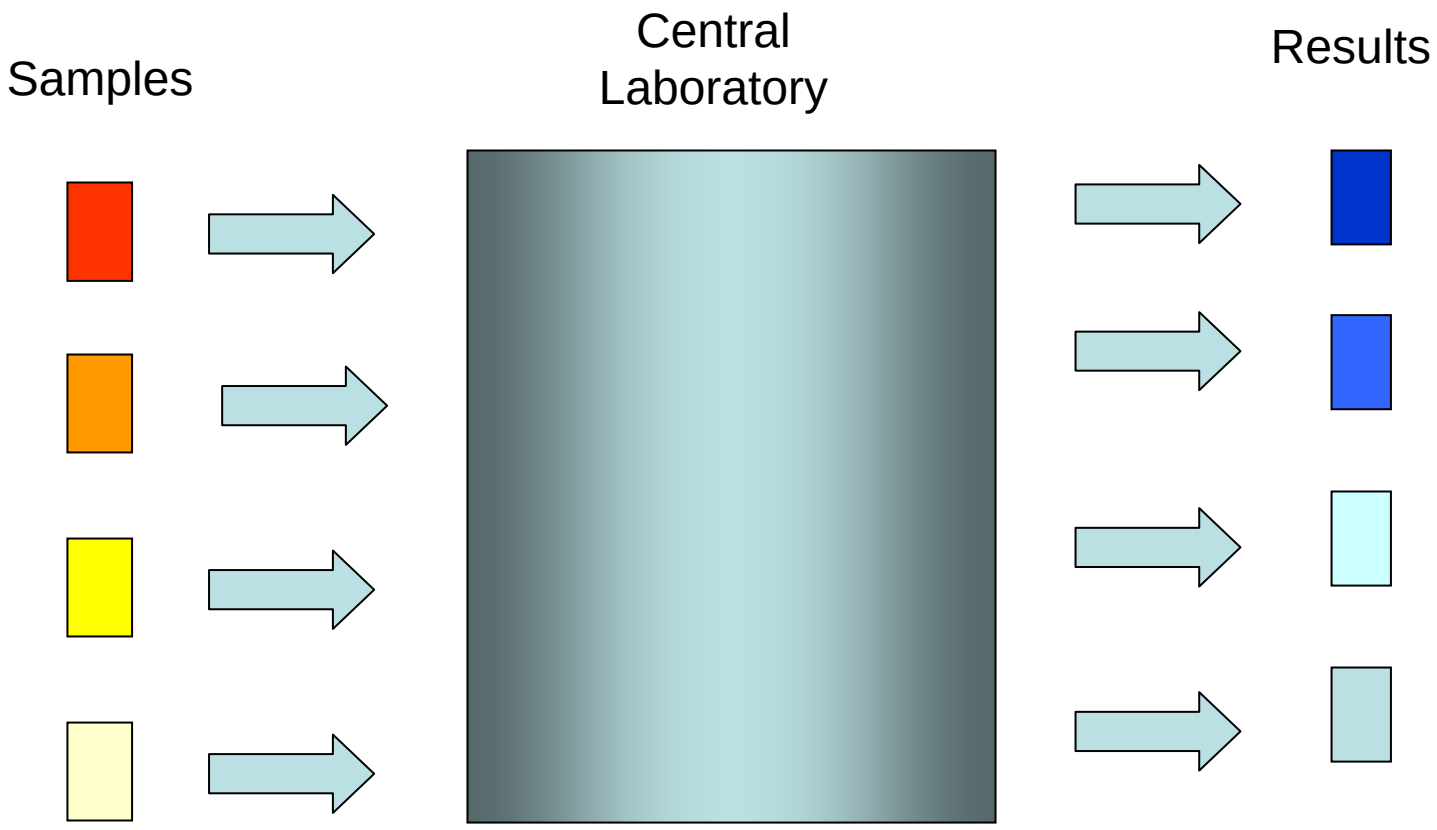


HIV Phenotypic Resistance Testing: Emergence of the Central Laboratory



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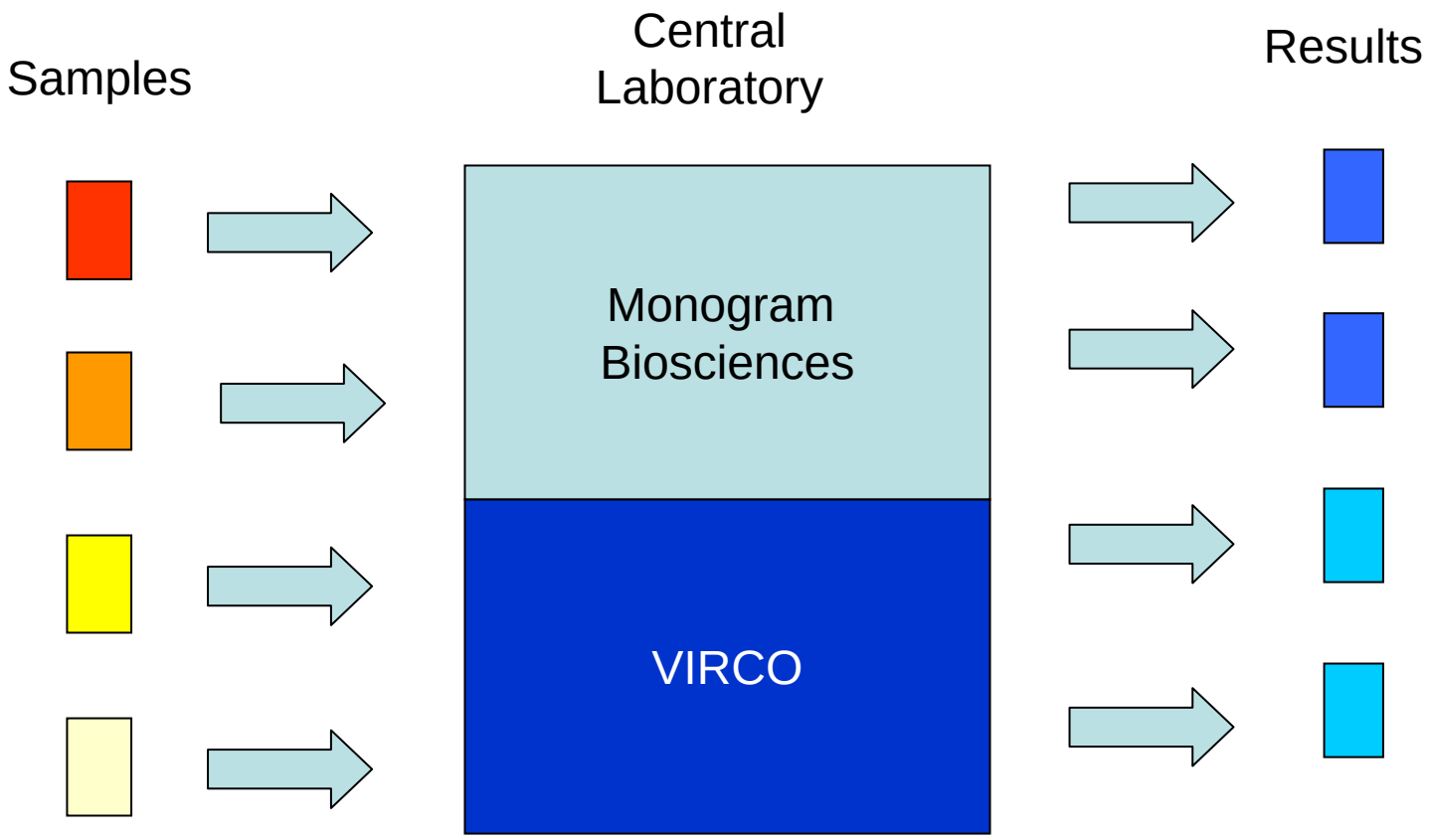

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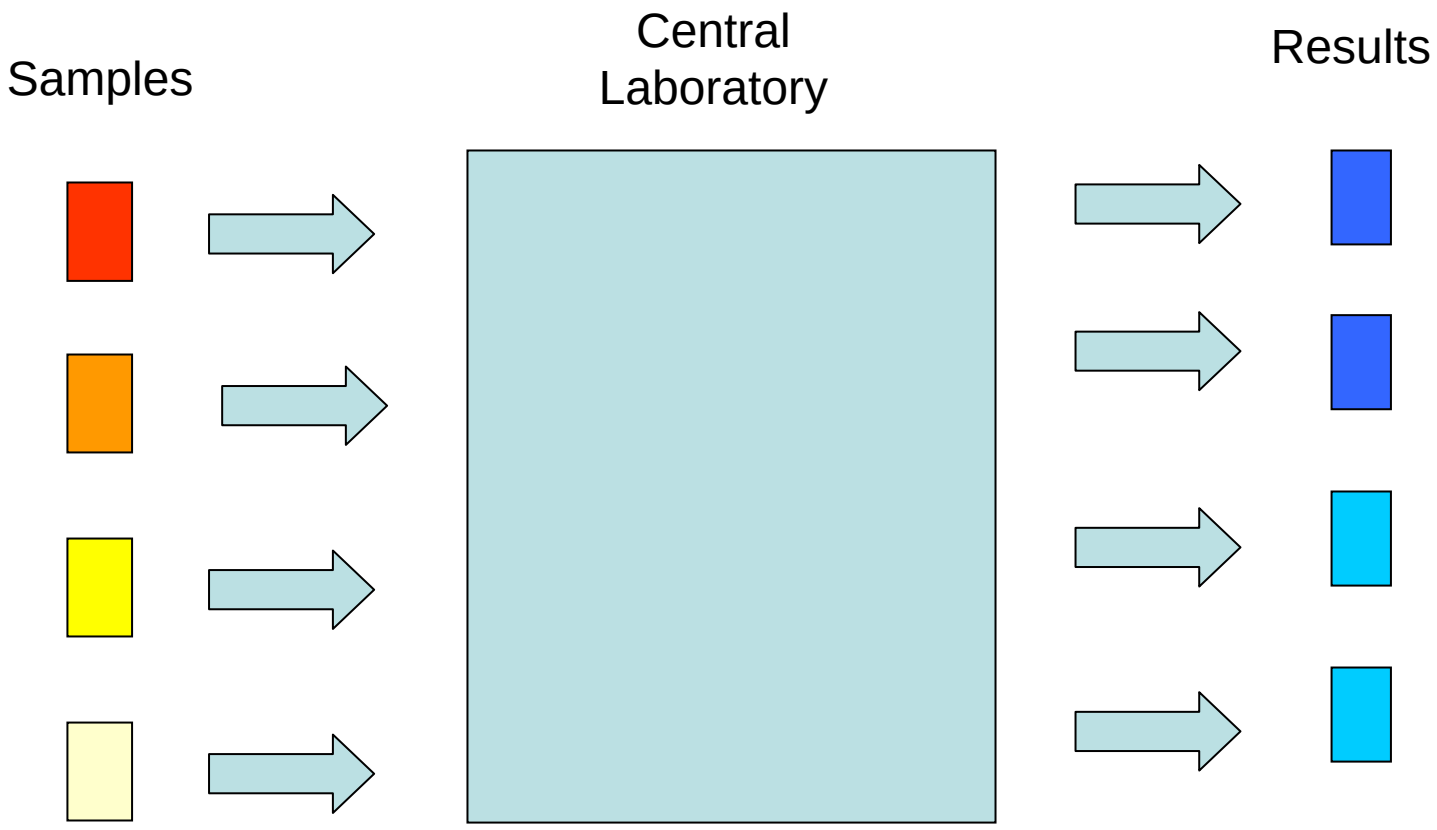


Multiple or Central Laboratories?

- Favors Multiple Laboratories
  - Fewer variables in assay performance
  - Fewer steps in process
  - Less need for precision
- Favors Central Laboratory Approach
  - Many variables affect assay results
  - Mechanization required for precision
  - Large capital investment required
  - High need for precision

HIV

- Viral Quantification
  - Mix of large commercial laboratories and smaller hospital and academic laboratories
    - commercial reagent kits
    - Iterative QA/QC through standardized approaches
- Resistance Testing
  - Almost exclusively central laboratory format
    - Requires mechanization for precision and efficiency
    - Too much capital investment for small scale laboratories

HCV Quantification

- Reasonably analogous to HIV
- Mix of large commercial and smaller hospital and academic laboratories

HCV Resistance Testing

- Genotypic testing
  - Much more background variability
  - Clonal analysis required
  - Genetic polymorphisms may have major impact on phenotype
  - Relationship between selection in replicons and in clinical strains less clear
- Phenotypic testing
  - Enzymatic assays require protein expression and are not likely applicable in clinical practice setting
  - No current cell culture system for in vitro cultivation of clinical isolates
    - Wakita strain: Genotype 2 and requires royalties if the virus touches a potentially commercial compound
  - Replicon recombinant systems not for the timid

Standardization of Laboratory Assays for HCV Drug Resistance: Opportunities and Challenges

- Laboratory issues
- Issues in discovery, clinical development and clinical practice

Issues in HCV Resistance Testing in Discovery and Development

- What do we want to know?
  - Preclinical Drug Development
    - How well do laboratory-derived susceptibility data mirror results derived from low passage clinical isolates?
    - How much variability is there when one studies a representative sampling of clinical strains
      - Within HCV subtypes
      - Between HCV subtypes
    - Enzymatic or cell culture based systems?
    - Does *in vitro* selection of replicons (or of full length virus) fully capture the behavior of clinical strains?

Issues in HCV Resistance Testing in Discovery and Development

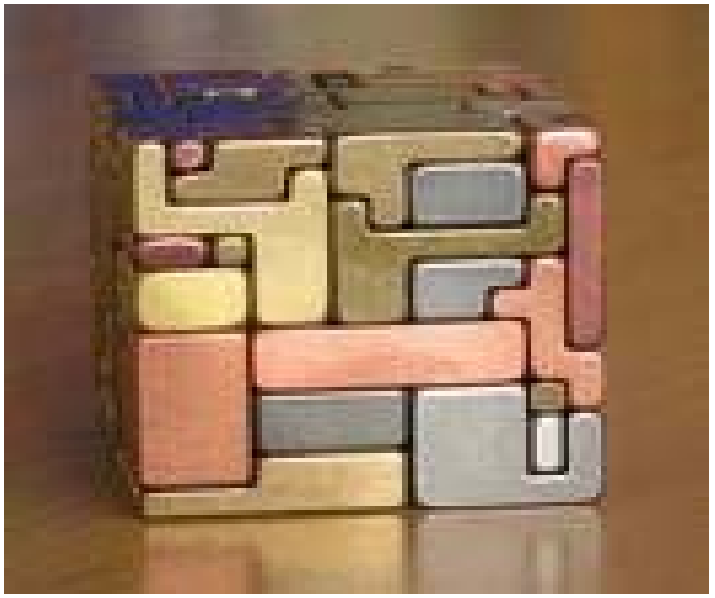
- What do we want to know?
  - In clinical trials
    - How does baseline susceptibility affect antiviral activity?
    - What are the kinetics of the appearance of viral strains with reduced susceptibility?
    - Does selection with test agent affect susceptibility to other agents in development?
    - What are the kinetics of replacement of drug resistant variants when selective pressure is removed?
    - Do we need to consider issues related to compartmentalization?
      - e.g. Hepatic vs. non-hepatic sites
    - Are there intra- or extra-hepatic reservoirs that will result in “archived” resistance?
    - Should expected endpoints be formally specified?

Selected Issues in HCV Resistance Testing in Clinical Practice

- What is the impact of HCV genotype?
- Is genotypic testing sufficiently predictive?
- Will baseline resistance testing be required because of inter-strain variability in susceptibility?
- If a regimen fails, what are the implications for use of these agents in subsequent regimens?
- Will drug resistant virus be transmitted?
- What is the impact of minority species variants?

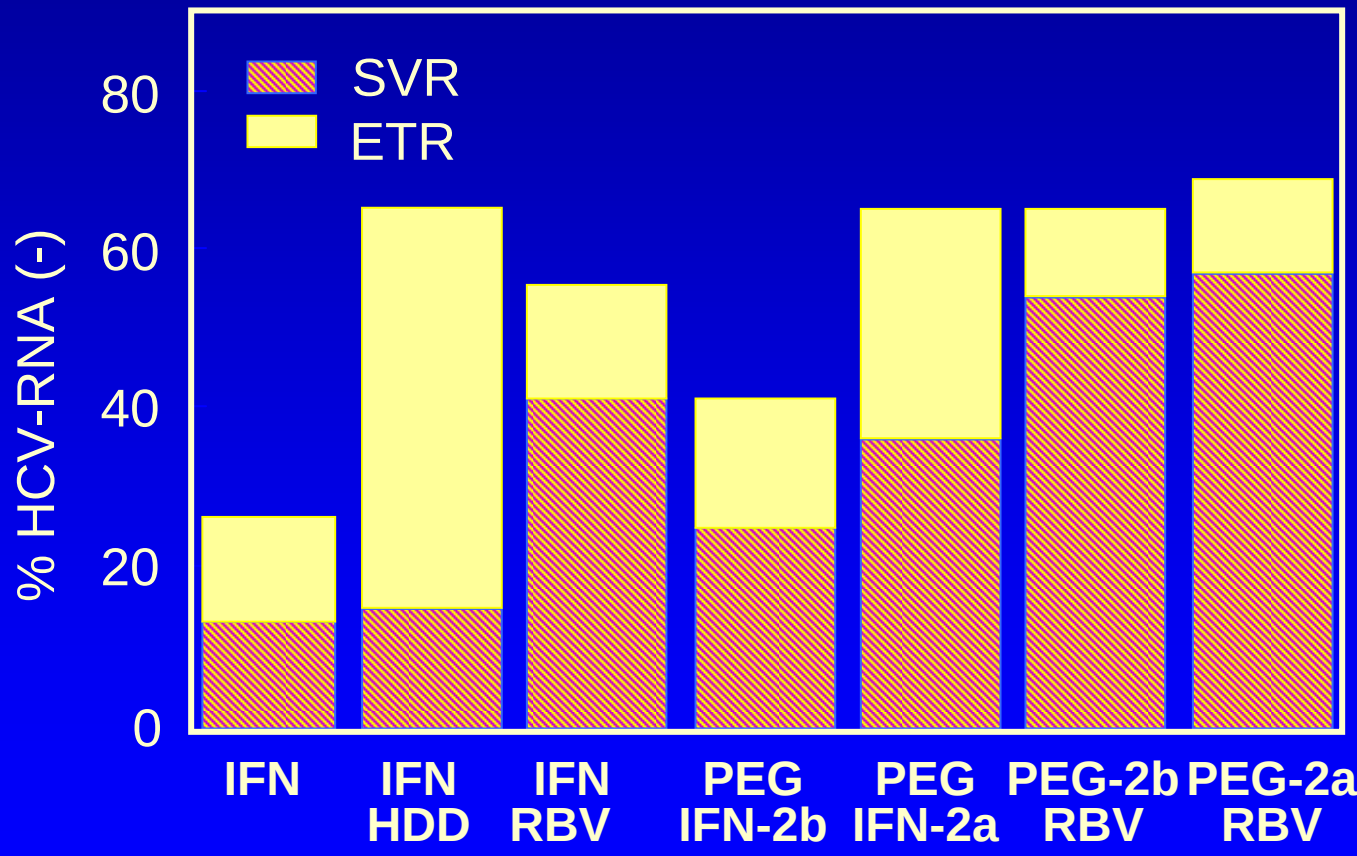
HCV: More Moving Parts than HIV

HIV



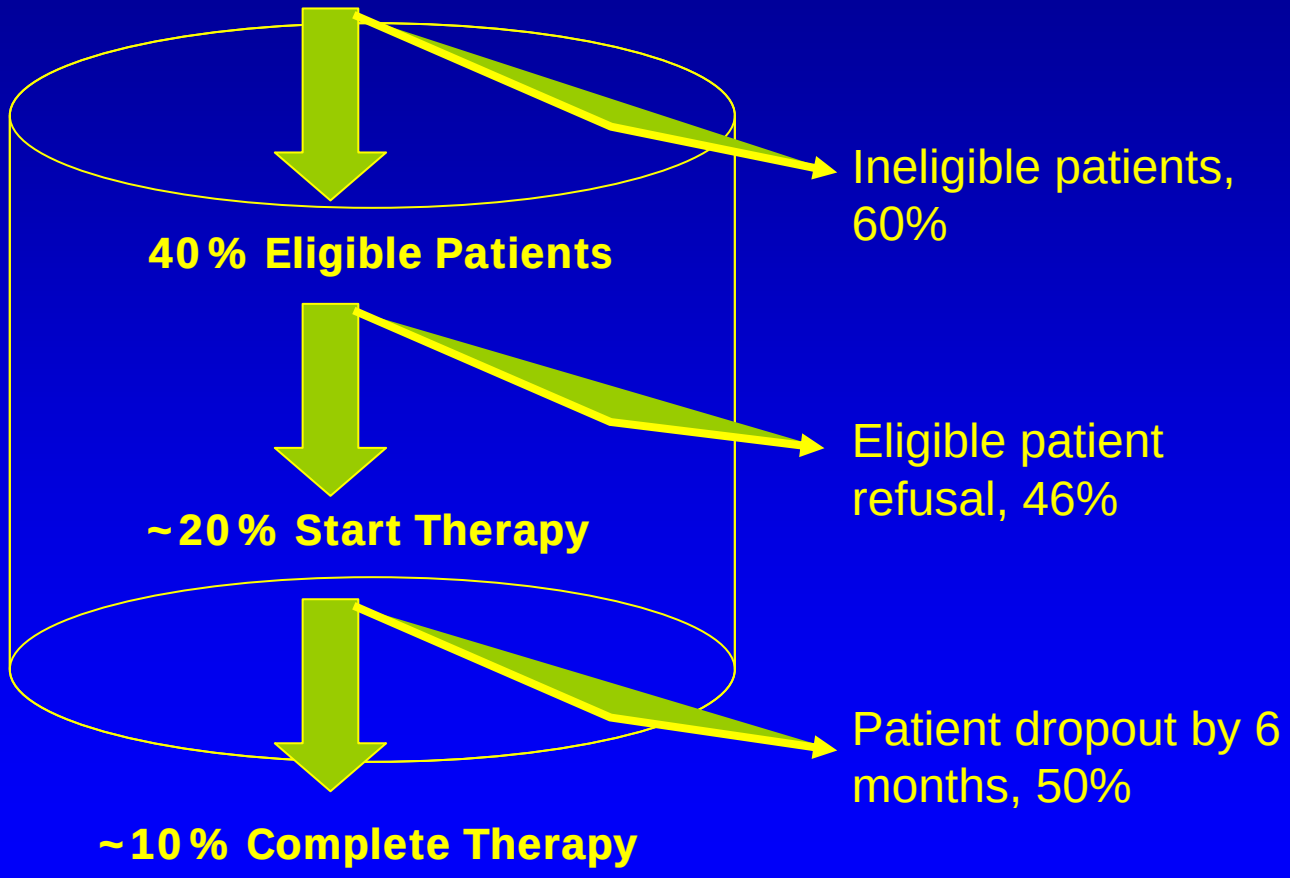
HCV

Evolution of Therapy for Hepatitis C Virus



Shiffman ML Clin Liver Dis 2001.

The Current Development/Regulatory Paradigm Focuses on the "Small Stuff"



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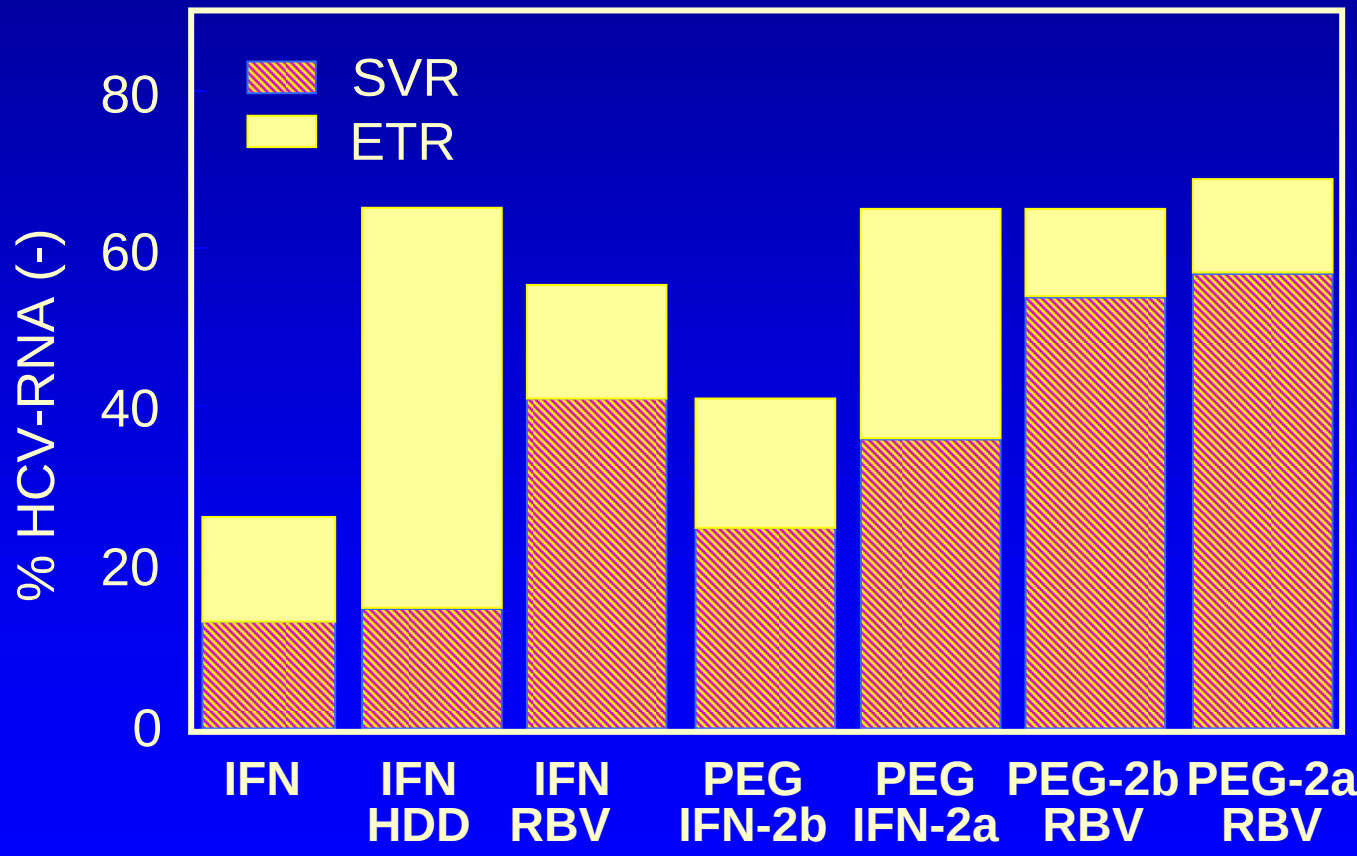

Shiffman ML Clin Liver Dis 2001.

Combination Regimens, Likely Constructed Across Company Lines Will be Required

Harmonization/Coordination Possibilities for HCV

- Sequence database
- Development of a “standard” clinical strain library that can be shared among those in drug development or laboratory
  - Components: Genotypes 1a and 1b and others, isolates from clinical trials
  - Possible formats: HIV RNA, expressed protein, subgenomic replicons
- Development of consensus procedures where feasible
- Open sharing of critical molecules from a common repository
- Development and support of central laboratory(ies) for provision of standardized assays
- Development of consensus approaches to clinical investigation and clinical trials endpoints