A Novel Approach to Sample Stabilization for Global Health Applications

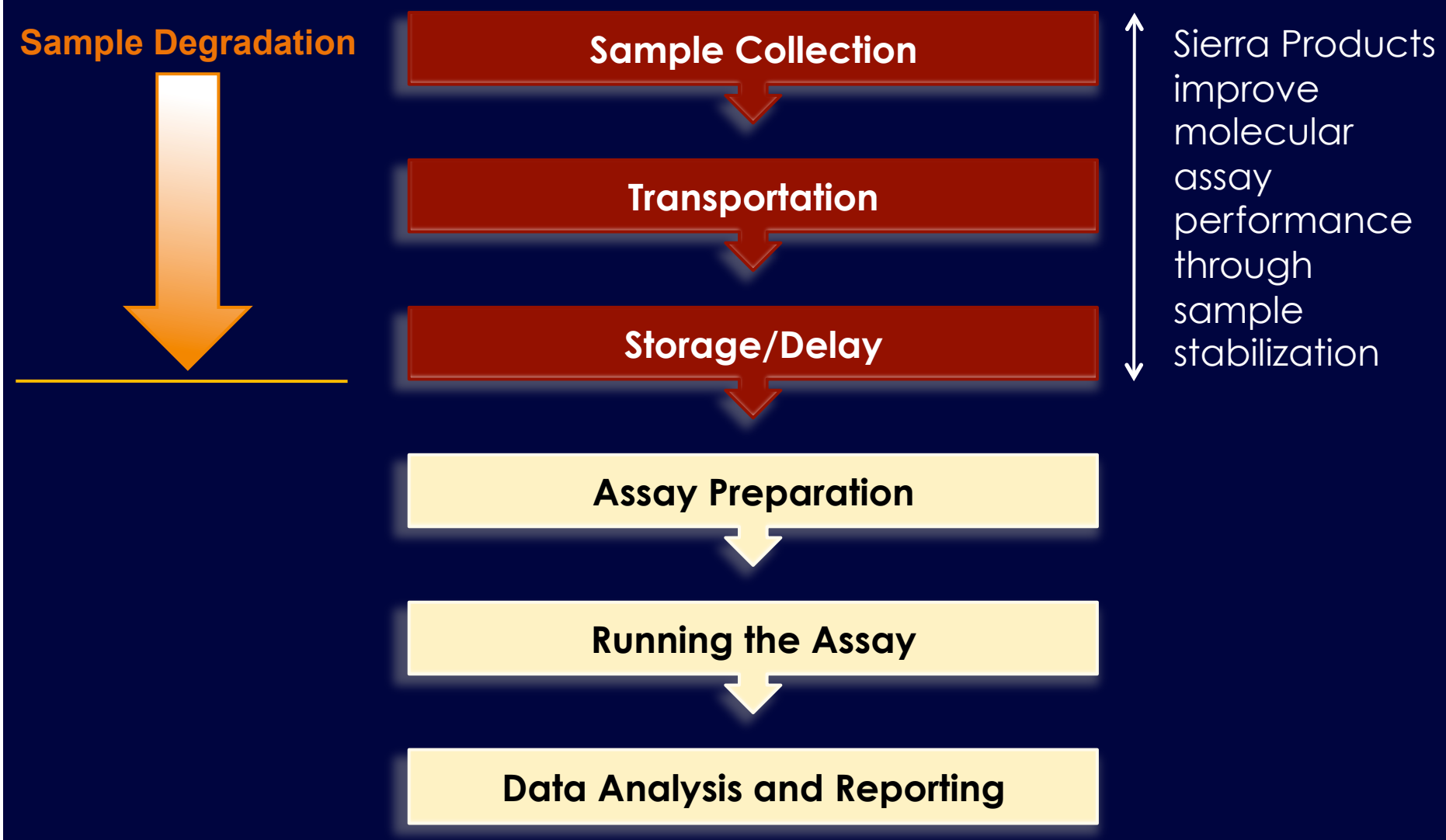
6 May 2010

US Centers for Disease Control and Prevention
International Laboratory Branch

I.

Introduction to Sierra Molecular's AssayAssure® Sample Stabilization Tools

From Sample to Information



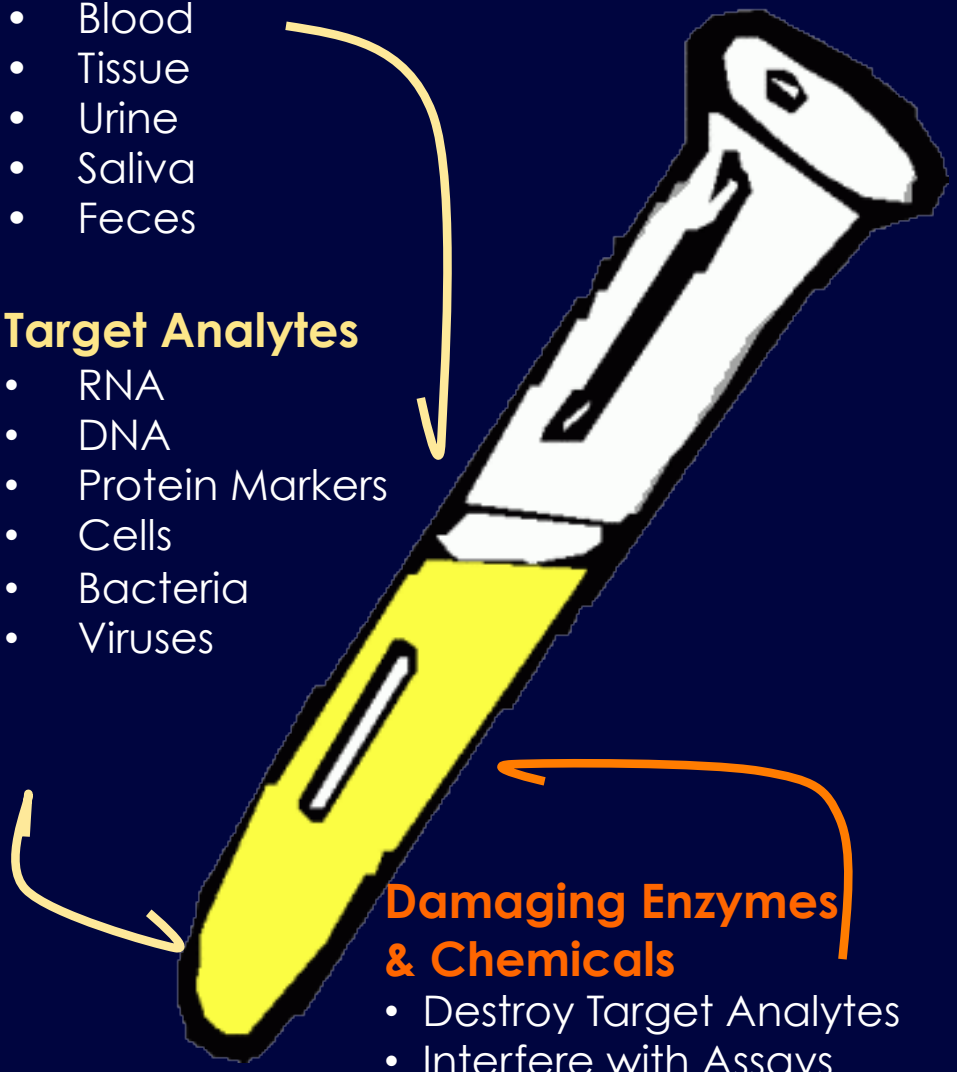
Technology Overview

Sample Matrices

- Blood
- Tissue
- Urine
- Saliva
- Feces

Target Analytes

- RNA
- DNA
- Protein Markers
- Cells
- Bacteria
- Viruses



Damaging Enzymes & Chemicals

- Destroy Target Analytes
- Interfere with Assays

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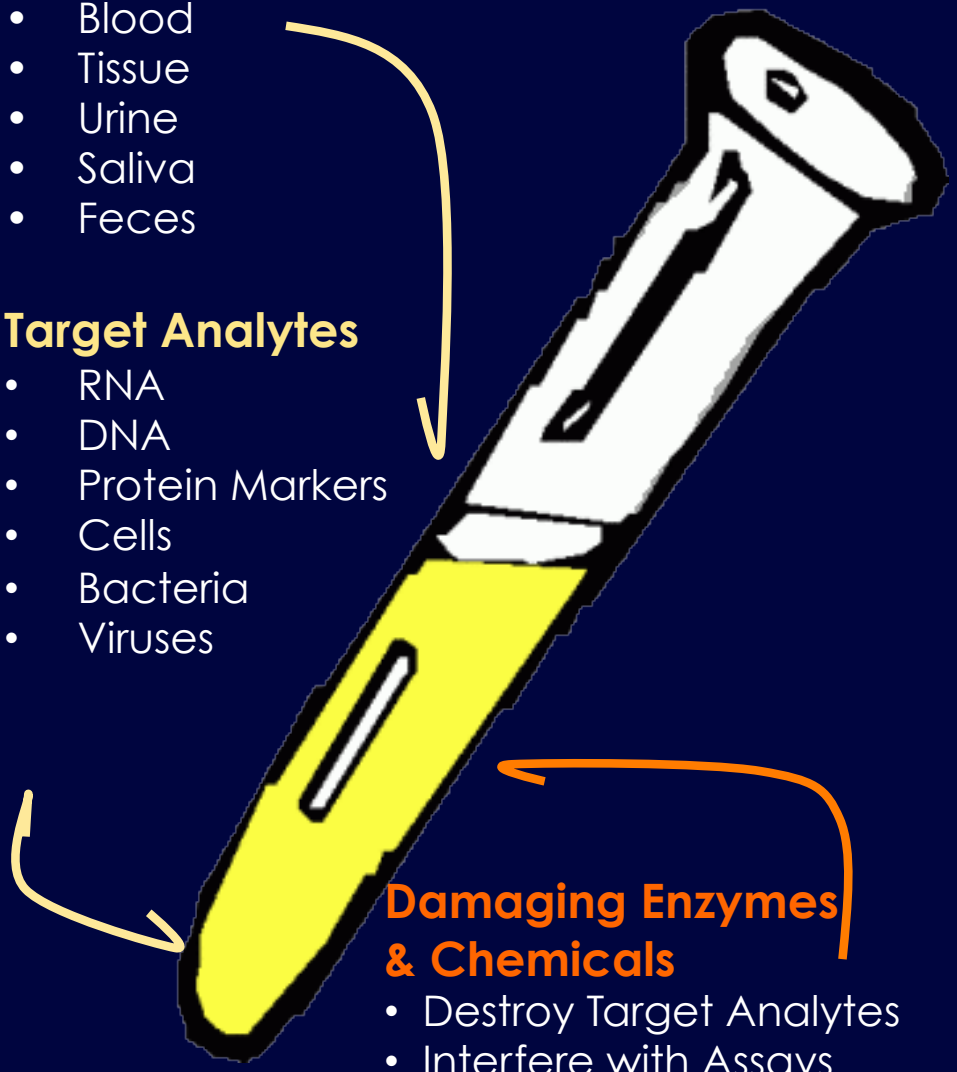

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The Sierra Molecular Solution

- Maintain cell viability
- Inactivate destructive chemical and biological agents
- Stabilize fragile target molecules

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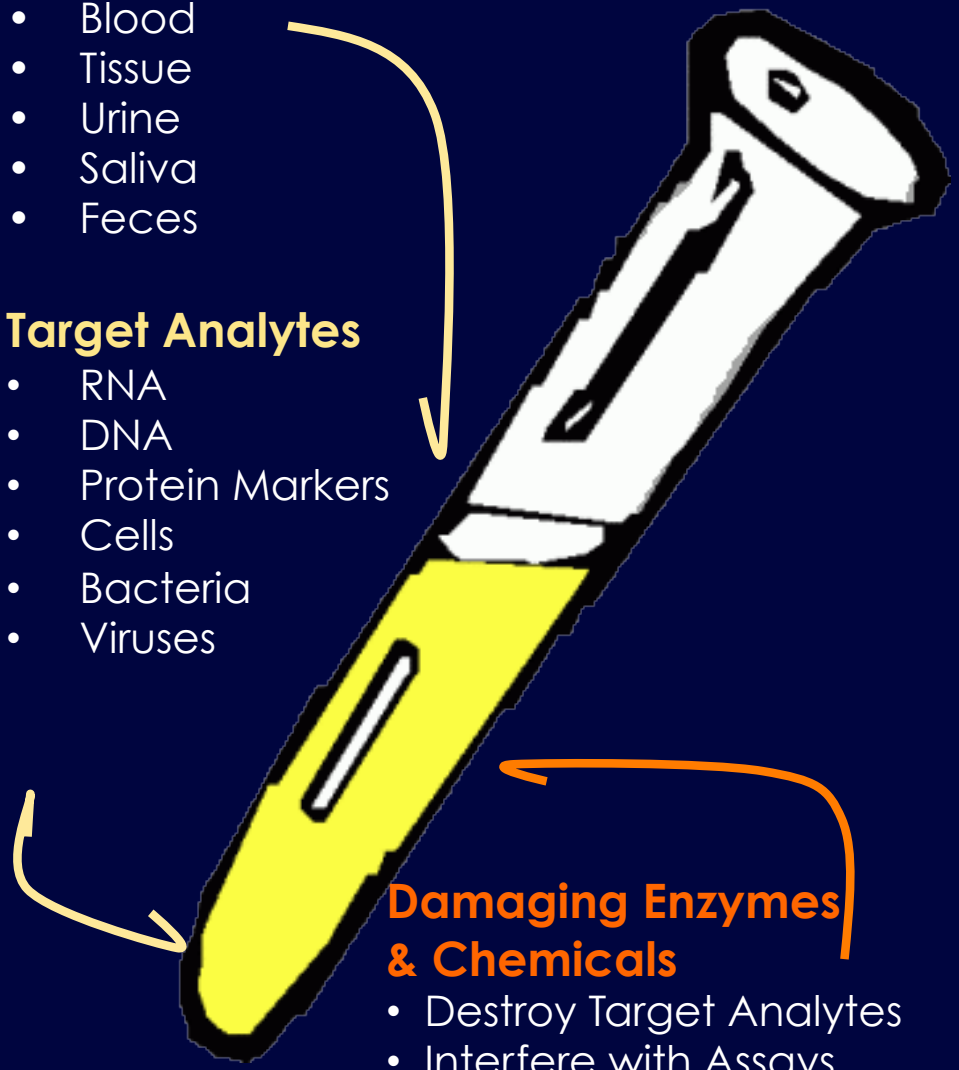

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Benefits of a "Chemical Refrigerator"
- Stabilization for days or weeks
- No refrigeration or freezing: eliminates logistical complexity and expense of cold-chain transportation and storage
- Simple laboratory techniques
- No proprietary sample preparation kits
- Permits molecular (PCR, qPCR, microarray), flow cytometry, and chemistry assays, as well as microbiology culture.
- Inexpensive

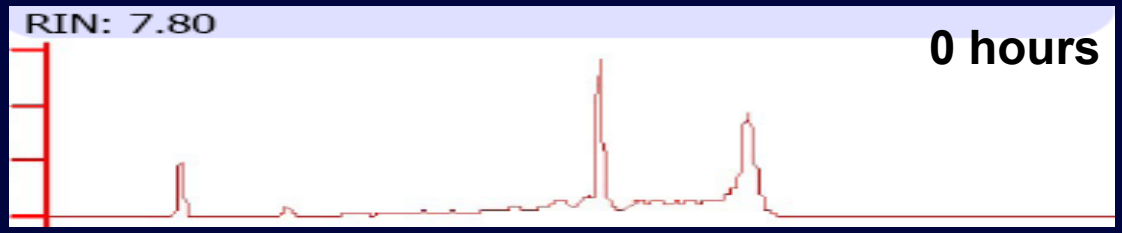
Mechanisms of Action

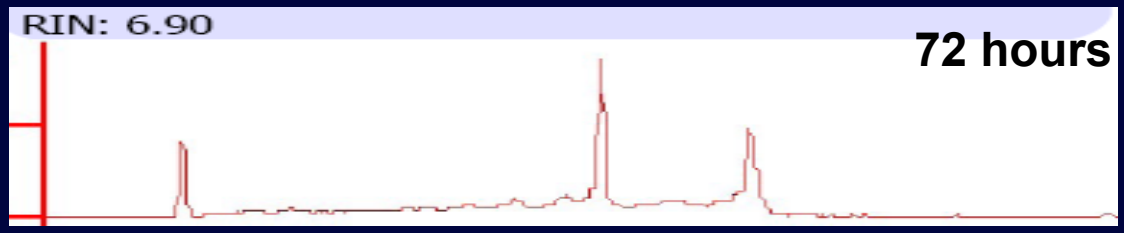
1. Prolong Cell Viability: slowing cellular metabolism and providing thermal protection.
2. Maintain Bacteriostasis: retarding the reproduction and lysis of bacteria, whose enzymes are highly destructive of genetic targets.
3. Remove Assay interferences: neutralizing the enzymes and proteins within the sample matrices, which damage Taq polymerase and thereby inhibit amplification.
4. Suppress Nuclease and Protease Activity: helping to preserve the most labile nucleic acids targets.

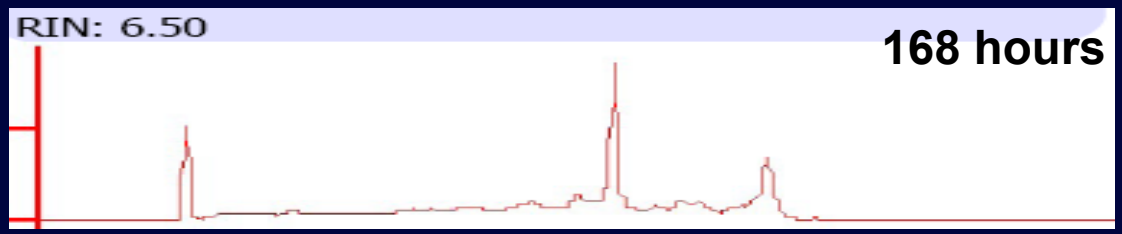
Nucleic Acid Preservation

By suppressing nuclease and protease activity and providing a degree of thermal protection, AssayAssure sample stabilization tools preserve RNA and DNA targets.

RNA Quality to168 Hours via Agilant Bioanalyzer

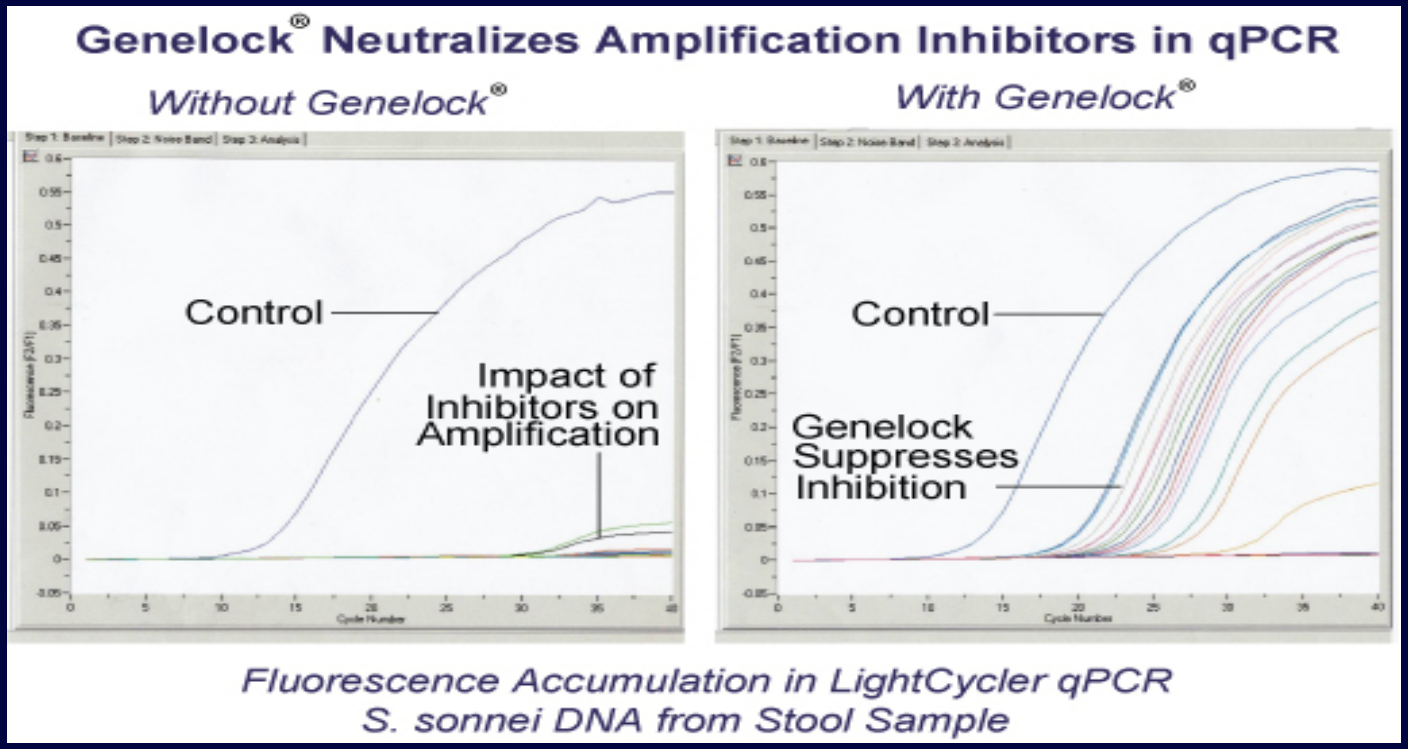






Neutralization of Amplification Inhibitors

By counteracting the inhibitory effects of certain enzymes and chemicals on Taq, AssayAssure allows better amplification with PCR, improving sensitivity and specificity of the assay.

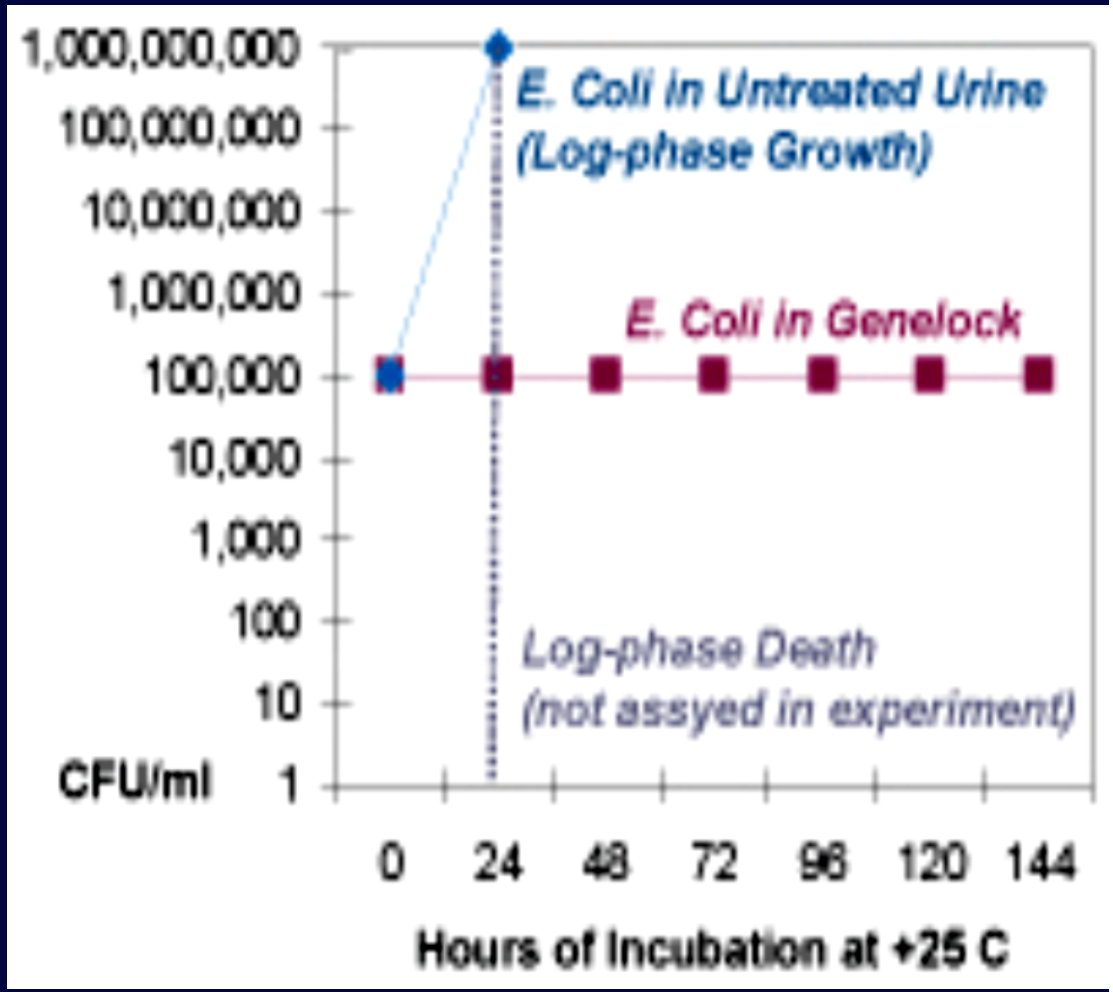


Bacteriostasis

AssayAssure stabilizes bacterial populations without refrigeration.

This permits identification and quantification of particular species within bacterial communities: Human Microbiome Project

Unlike culture media, AssayAssure prevents both bacterial death and overgrowth.



Cell Viability

Cellular Viability Assessment

Stabilization Method & Sample Aging	Cellular Clumps	Viability: Trypan Blue Exclusion	Viability: CD45/PI
EDTA – Day 1	None Detected	Cells Viable	99.23%
AssayAssure® - Day 1	None Detected	Cells Viable	99.70%
AssayAssure® - Day 8	None Detected	Cells Viable	94.94%

Hematology

Stabilization Method & Sample Aging	Leukocyte Count (WBC) ($x10.e^3/\mu L$)	Erythrocyte Count (RBC) ($x10.e^6/\mu L$)	Hemoglobin Concentration (HGB) (g/dL)	Hematocrit Value (HCT) (%)
EDTA – Day 1	6.11	4.85	14.57	40.10
AssayAssure® - Day 1	5.43	4.26	12.90	35.25
AssayAssure® - Day 8	4.06	4.24	12.70	37.70

Stabilization Method & Sample Aging	Mean Corpuscular Volume (MCV) (fL)	Mean Corpuscular Hemoglobin (MCH) (pg)	Mean Corpuscular Hemoglobin Concentration (MCH) (g/dL)	Red Cell Distribution Width (RDW) (%)	Platelet Count (PLT) ($x10.e^3/\mu L$)	Mean Platelet Volume (MPV) (fL)
EDTA – Day 1	82.70	30.03	36.37	13.37	229.00	11.47
AssayAssure® - Day 1	82.85	30.25	36.55	13.25	92.50	10.50
AssayAssure® - Day 8	88.80	29.9	33.7	13.00	89.00	13.30

Stabilization Method & Sample Aging	Total Neutrophils (# NEUT) ($x10.e^3/\mu L$)	Total Lymphocytes (# LYMPH) ($x10.e^3/\mu L$)	Total Monocytes (# MONO) ($x10.e^3/\mu L$)	Total Eosinophils (# EOS) ($x10.e^3/\mu L$)	Total Basophils (# BASO) ($x10.e^3/\mu L$)	Percentage Lymphocytes (% RECTIC) (%)	Total Reticulocytes (# RETIC) ($x10.e^6/\mu L$)
EDTA – Day 1	3.83	1.70	0.34	0.08	0.03	1.37	0.07
AssayAssure® - Day 1	3.24	1.52	0.45	0.11	0.02	1.50	0.06
AssayAssure® - Day 8	2.34	1.06	0.38	0.07	0.12	0.80	0.03

Cell Viability

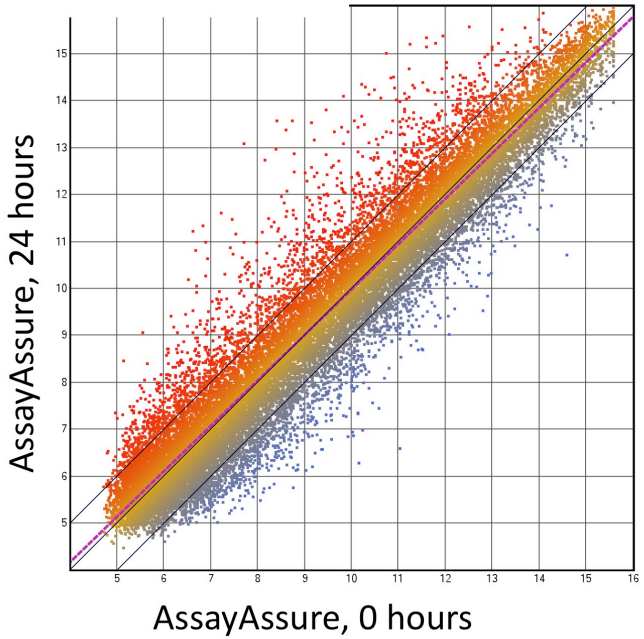
By keeping lymphocytes viable and limiting hemolysis, AssayAssure stabilizes whole blood samples for immunophentyping applications, including flow cytometry.

Cell viability also improves the quality and yield of mRNA from blood samples.

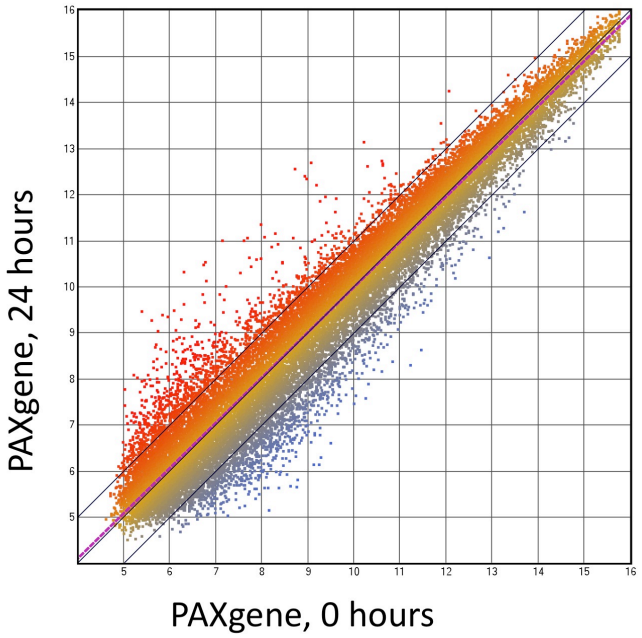
Gene Expression Stabilization

AssayAssure was compared against PAXgene at 0, 24, 48, 72, and 96 hours over 24,000 genes on a cDNA microarray.

AssayAssure
Pairwise Correlation= .962
24,000 genes



PAXgene
Pairwise Correlation= .971
24,000 genes



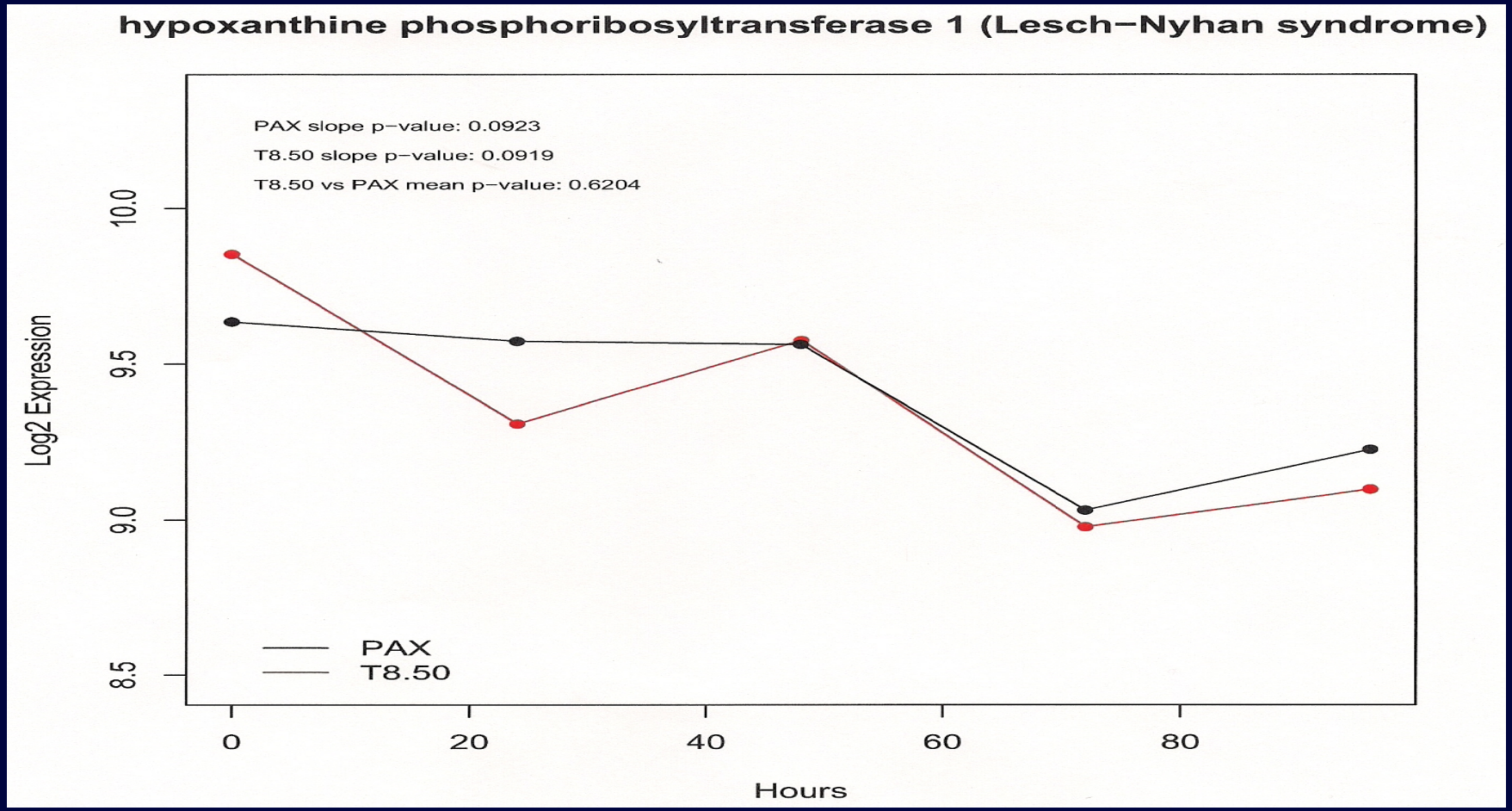
NimbleGen
HIGH - DEFINITION GENOMICS™

Methods: 1) Blood samples from a healthy donor were processed with PAXgene or incubated with AssayAssure for up to 96 hours; 2) RNA from white blood cells was purified from AssayAssure blood samples using, Nimbleen. LeukoLock (Ambion); 3) Global gene expression was assayed using NimbleGen Microrrays.

Source: ARUP Laboratories

Gene Expression Stabilization

AssayAssure v. PaxGene with HPRT Housekeeping Gene



AssayAssure® Product Formats

Hemolock Blood Stabilization
- Molecular Whole Blood Stabilization Tube
- Universal HIV Whole Blood Tube

Genelock Urine Stabilization
- Urine Cups
- 4ml & 15ml Tubes
- Bulk Chemistry

Genelock Tissue Stabilization
- Tissue Sample Cups

Genelock Multiplex Formula
- 250 ml Bulk Chemistry
- 5ml Tubes

Genelock Swab Kits
- Standard Swab Kit
- Nasopharyngeal Swab Kit

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Public Health Collaborations

Providing Sample Stabilization for CDC Global Health Studies

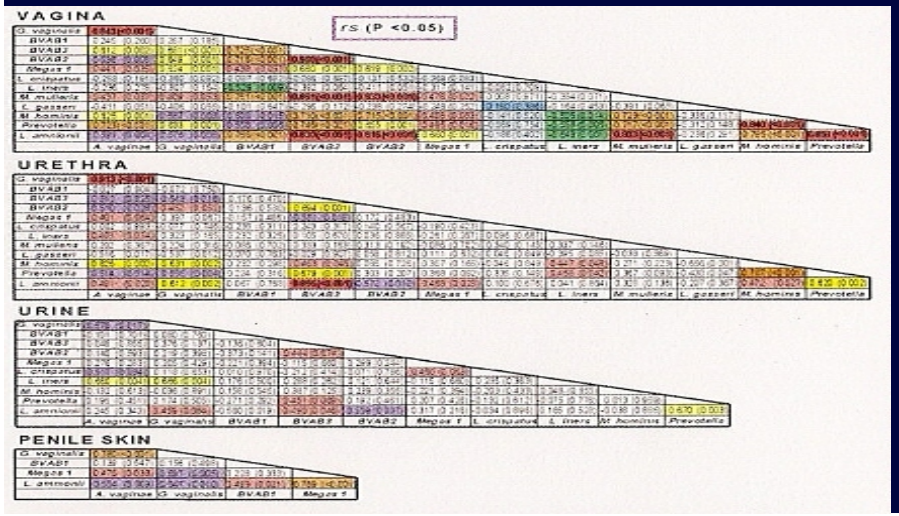
CDC researchers have used AssayAssure® chemistry for stabilization of molecular targets in several bench experiments and field studies:

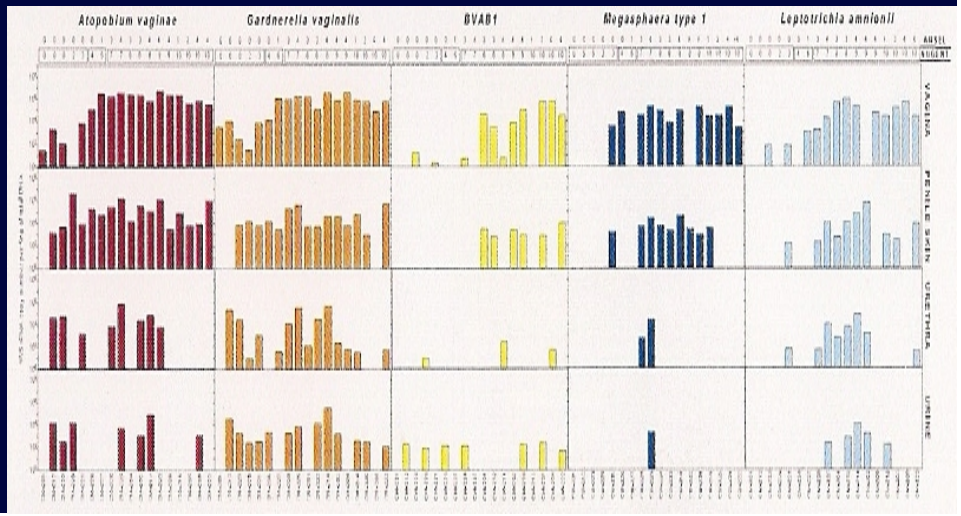
- Shigella Paper
- Thailand HIV/HSV Study
- Honduras ? STD Study

Providing Multiplex Stabilization to Human Microbiome Project

STD researchers at LSU (David Martin, et al.) and Staten Serum Institute (Skaagen-Jensen, et al.) are using AssayAssure to map communities of genital bacteria using multiplex qPCR.

They are finding new associations between imbalances between bacterial populations and previously idiopathic disease, and tracing transmission pathways.





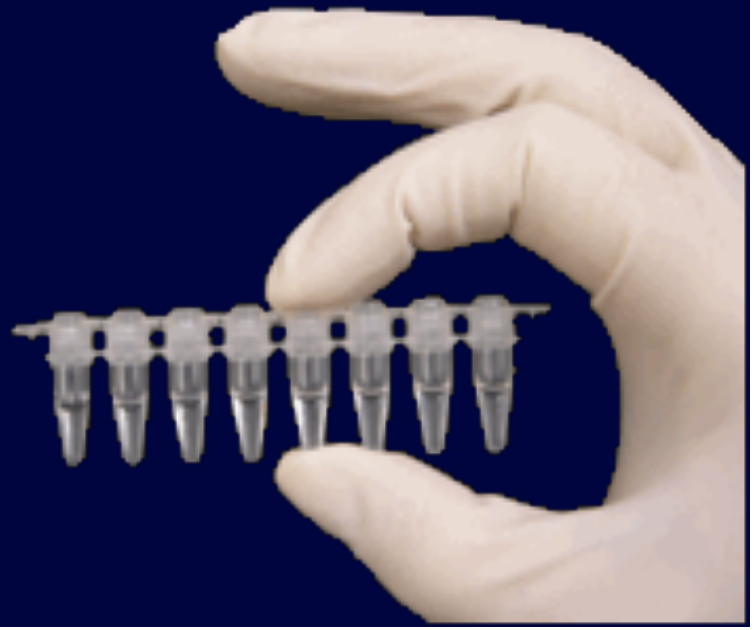
Stabilizing Circulating Gene Transcripts for Prostate Cancer Studies

Extremely labile, low-prevalence biomarkers from prostate cancer cells are found in urine samples stabilized with AssayAssure®:

- University of Michigan (Chinnayan Lab) – primary diagnostics
- Fred Hutchinson Cancer Institute – companion diagnostics
- Unilabs (Sweden) – primary diagnostics

STD Sample Stabilization for Molecular Assays for Public Health Agencies

AssayAssure® Chas been used by public health agencies and Planned Parenthood clinics throughout the US for improving target preservation in PCR assays for Ct/Ng.



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Future Applications in Global Health

Swab Kits

Universal Swab Kit

- Using Genelock sample stabilization chemistry, configured in Sierra Molecular's chemistry-in-the-sponge format
- Applications include food hygiene, bioterrorism assessment, and other environmental sampling

Nasopharyngeal Swab Kit

- Flu swabs
- FDA Class 1 exempt device, but requires flu-specific efficacy data

Swab Kits

Molecular Diagnostics Swab Kit

- Applications include STDs and topical infections
- FDA 510K approval necessary for assay-specific designation, probably not for general usage

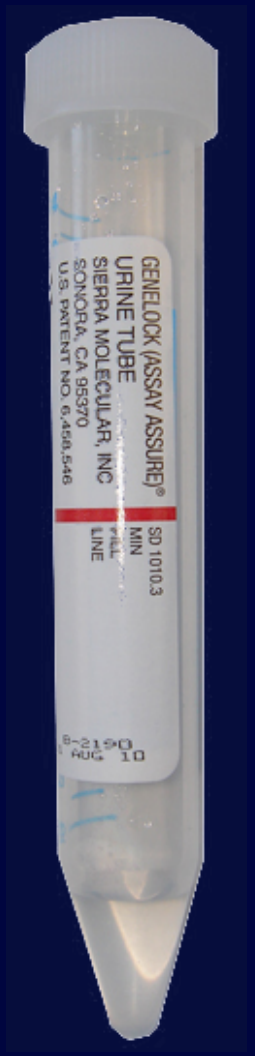
Genelock Multiplex Swab Kit

- Using Genelock Mulitplex sample stabilization chemistry in a liquid buffer format
- Wide-ranging research applications

Urine Tubes

Molecular Urine Tubes

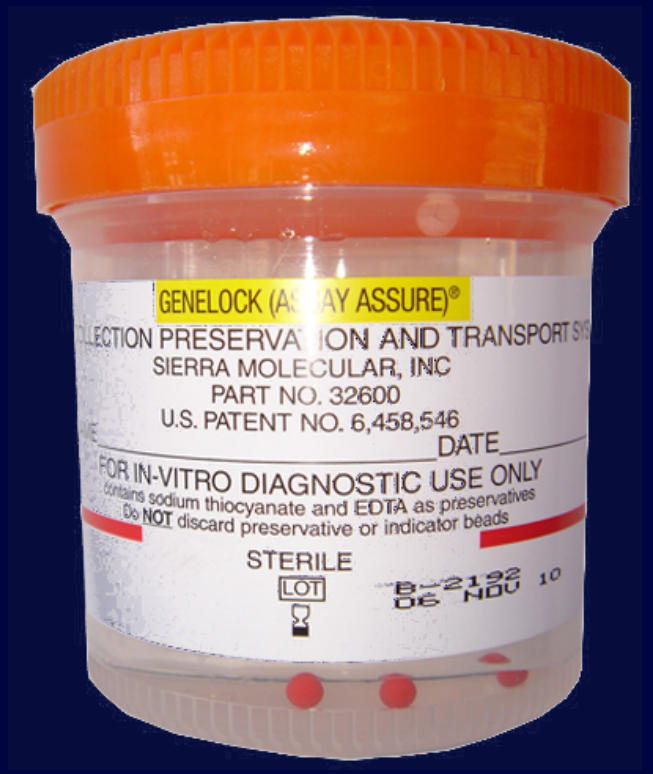
- This would be a new product offering using existing clear urine tubes
- Incorporating tableted Genelock chemistry
- Advantages: validated preservation of low-prevalence RNA/DNA targets and removal of molecular assay interferences



Urine Cups

Urine Cups for Dipstick Chemistry

- Incorporating tableted Genelock chemistry
- Advantages: suppresses bacterial growth; stabilizes pH; protects low target analytes; facilitates routine urine chemistry, microbiology, and all molecular testing



Tissue Collection Cups

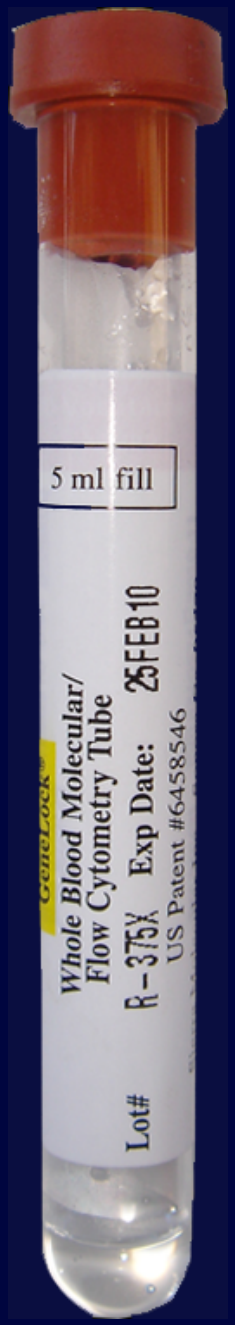
Cups for Collection and Stabilization of Tissue

- Incorporating Genelock Tissue chemistry in liquid format, to replace formalin
- Validation studies are currently underway
- Assay-specific applications would require FDA 510K certification
- Advantages: gives pathologist the ability to do molecular analysis as well as standard histology, without the toxicity of formalin

HIV Whole Blood Tube

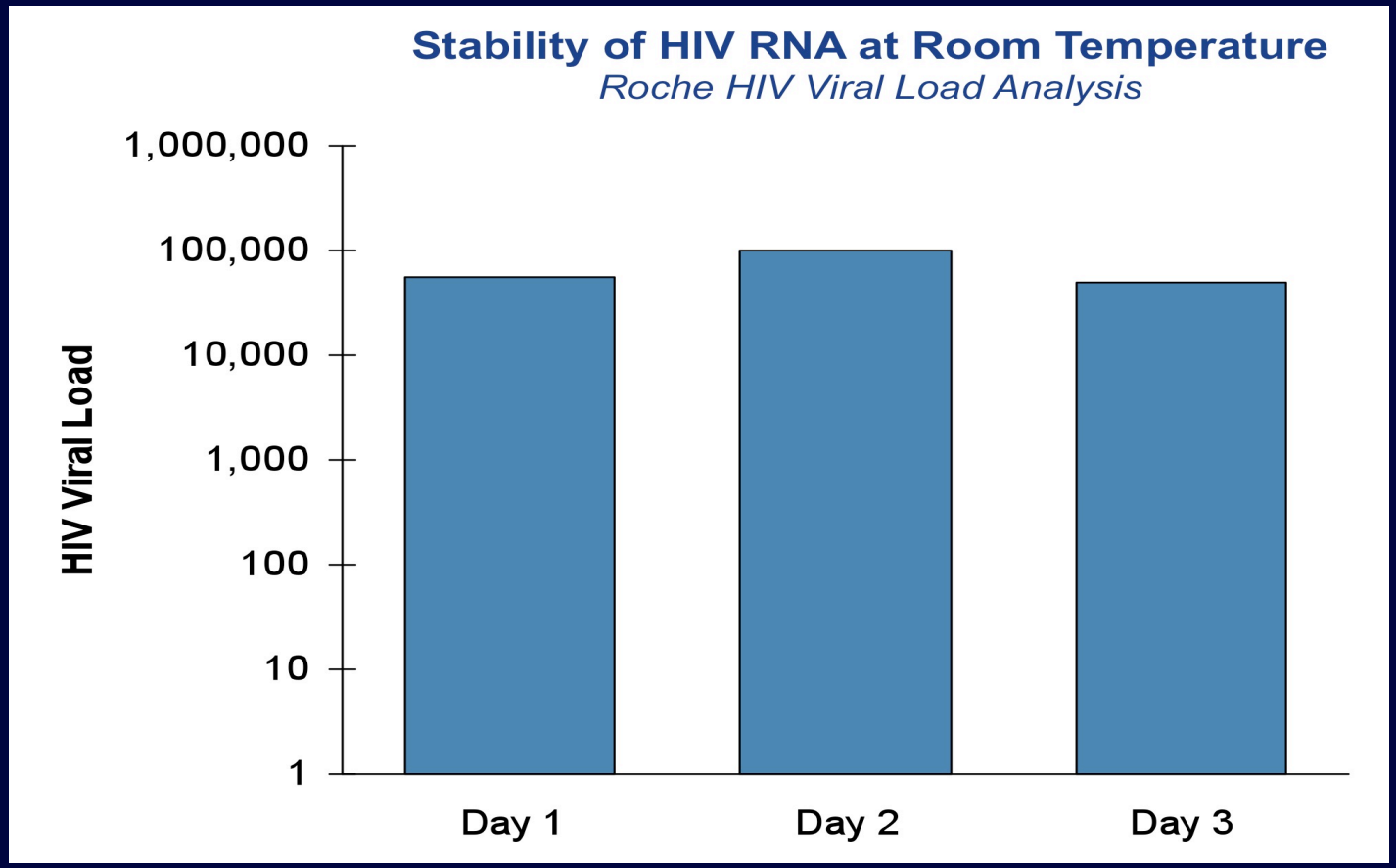
Single Tube for Collecting Whole Blood Specimens for monitoring ARV Therapy

- Primary detection, genomic viral subtyping, viral load quantification, and immunophentyping from one tube.
- No fractioning of blood necessary
- Unrefrigerated collection, transport, and handling up to seven days
- Advantages: gives pathologist the ability to do molecular analysis as well as standard histology, without the toxicity of formalin



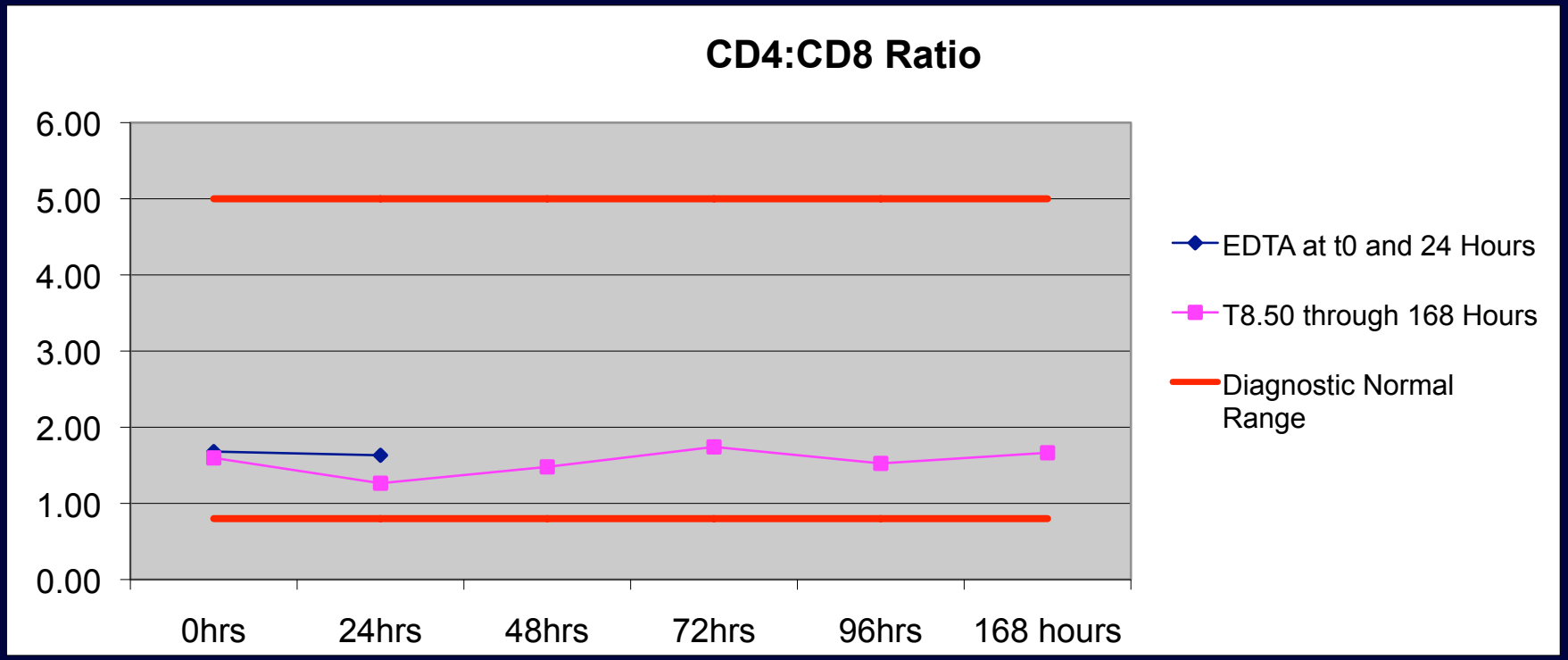
Sample Stabilization for HIV Viral Load Quantification

AssayAssure stabilizes RNA for consistent, reliable viral load measurements, over time, unrefrigerated.



Sample Stabilization for CD4:CD8 Immunophenotyping

AssayAssure was compared the "gold standard" (EDTA not to exceed 24 hours) using standard HIV lymphocyte subset panels in flow cytometry assays.



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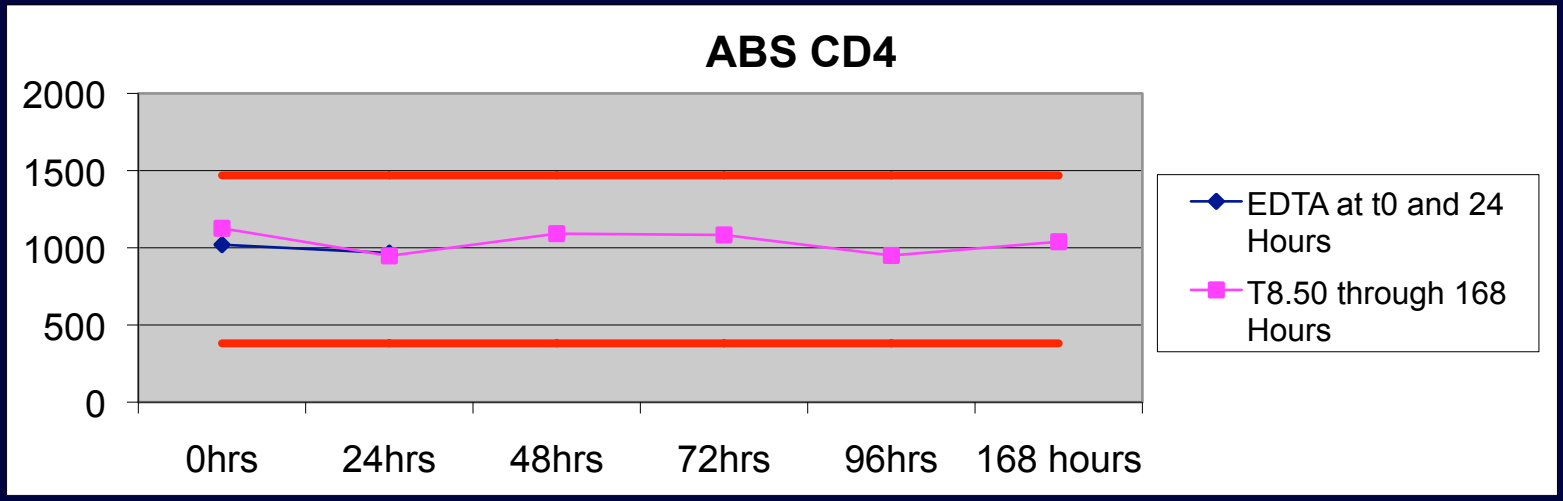

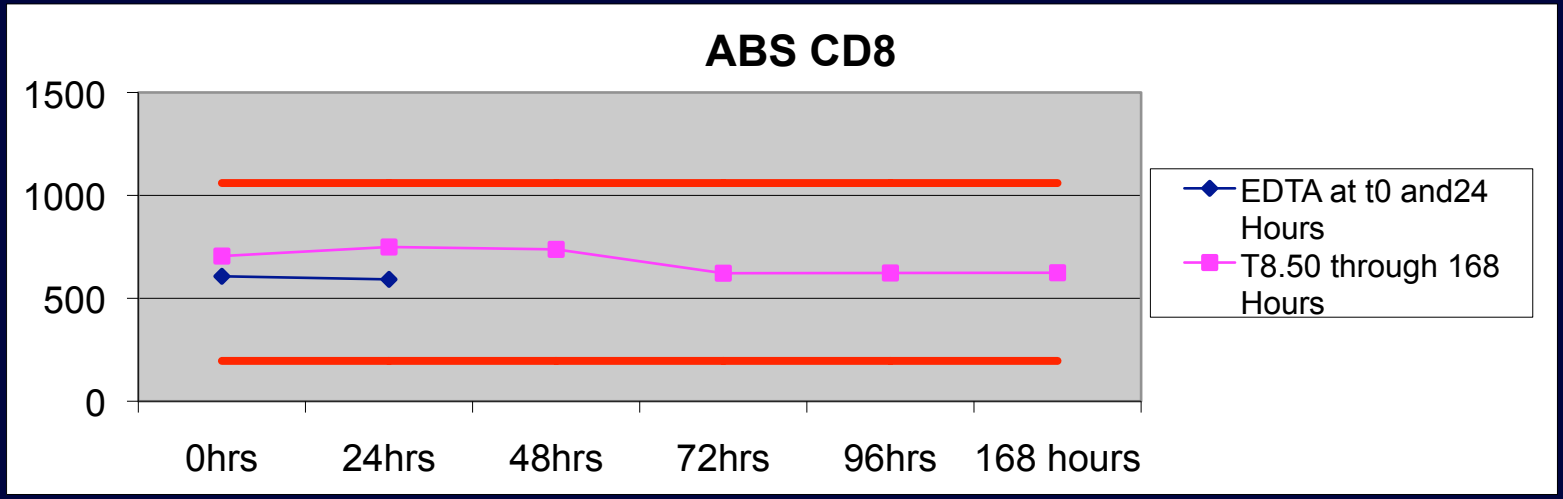


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