

Controls for Human Papilloma Virus (HPV) DNA testing

Mark Manak, Ph.D.
Boston Biomedica, Inc.

SoGAT XVII
Paris, France
May 27, 2004

Human Papilloma Virus Infections

One of most common sexually transmitted viruses

- 5.5 million new infections in US per year
- Most common cause of mortality
from gynecological malignancy world wide

80% of population will be infected with HPV in their lifetime.

- 28-46% by age 25.
- Most infections spontaneously cleared,
- 5-10% of women over 30 are persistent carriers of HPV
- 15,000 US women progress to cervical cancer annually
- 5,000 die

Fully preventable if detected and treated early

Detected by PAP smear

FDA approved combination of cervical cytology and HPV DNA testing for primary screening for women aged 30 yr and older

Cervical Cytology/HPV DNA Testing

HPV Sample Collection and Testing

Cervical Swabs

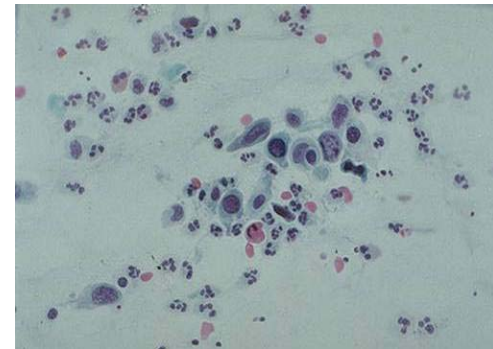
Used for both cytology and HPV DNA testing



Cell Morphology

%HPV DNA +

Normal	10%
ASC-US Atypical squamous cells of undetermined significance	20-40%
LSIL Low grade squamous intraepithelial lesions	60-85%
HSIL High grade squamous intraepithelial lesions	80-90%
Squamous Cell Carcinoma	99%



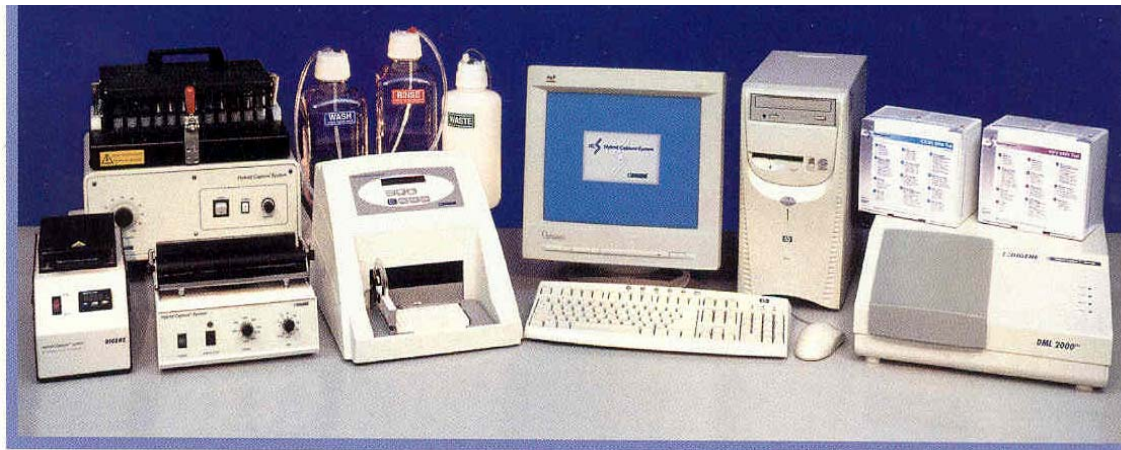
Digene's Hybrid Capture 2 HPV DNA Test

Hybrid Capture of DNA-RNA hybrids: MAb Detection

Uses two RNA probe cocktails

Differentiate between high and low risk HPV types

- High Risk Subtypes
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68
- Low Risk Subtypes
6, 11, 42, 43 44



From Digene Web site

ACCURUN 370: HPV DNA Control

Convenient Liquid Cytology “Liquid Pap Smear” Sample

- Packaged in Centrifuge Tube
- Processing identical to Clinical Sample
- In 4 ml PreservCyt or 2 ml SurePath

Challenging, yet consistent low level HPV Control

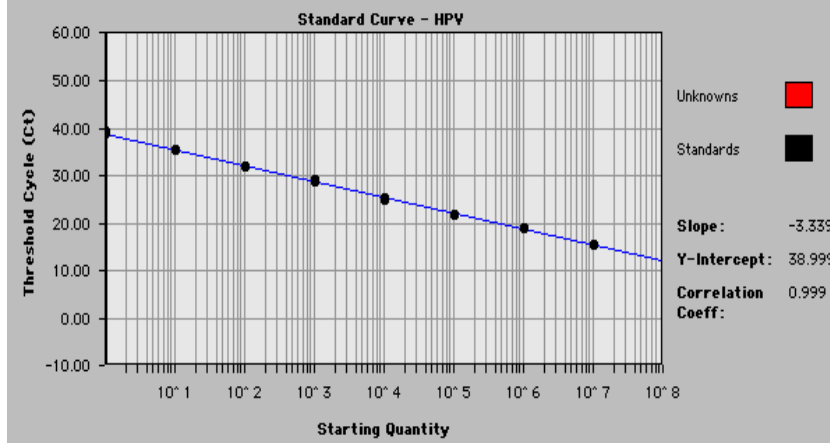
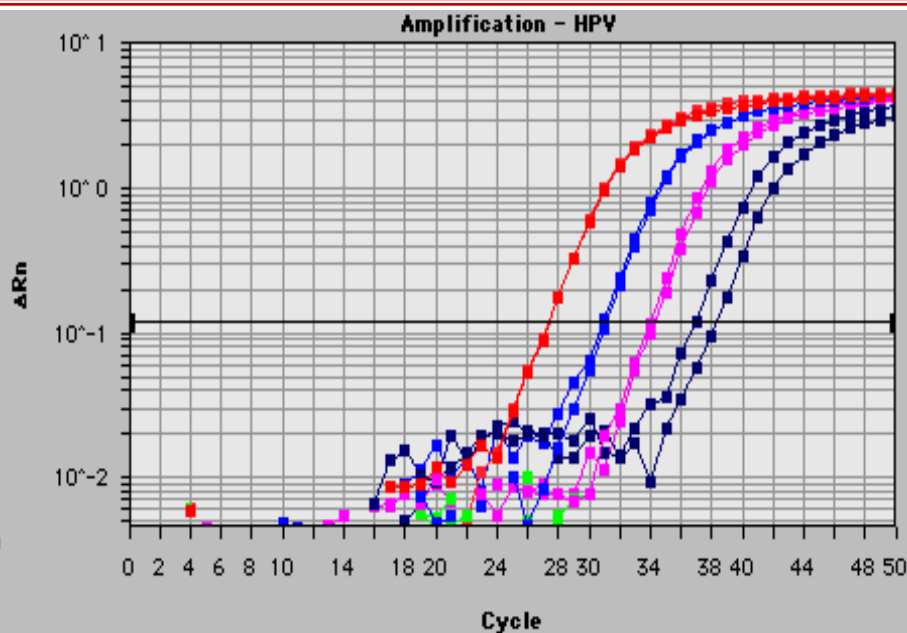
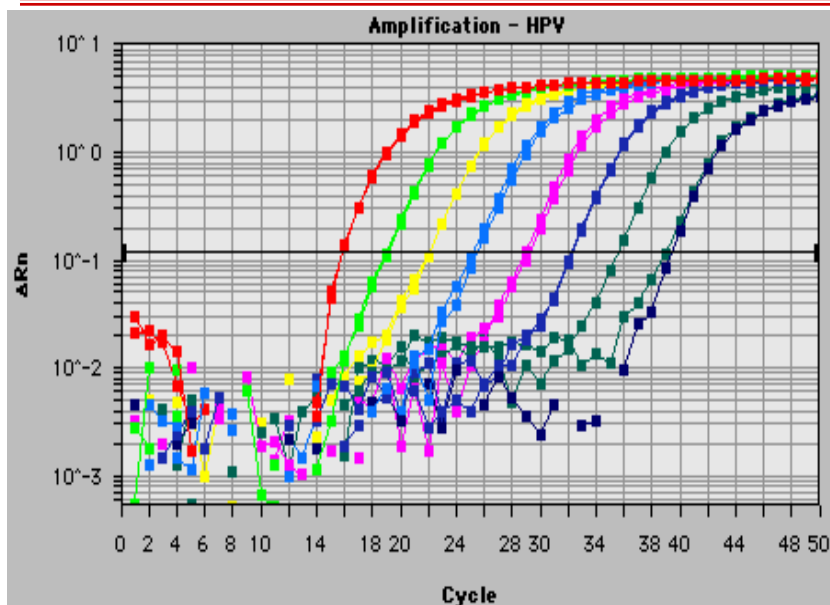
- Cultured human epithelial diluted with excess non-infected human cells
- Positive cells: 1-2 copies/cell of HPV Type 16 DNA
- HPV-16 most common high risk subtype in the US

Thoroughly characterized and quantitated

- Cell Count, hemocytometer and Guava
- Cell staining specific for SiHa cells
- Digene HC2
- HPV TaqMan

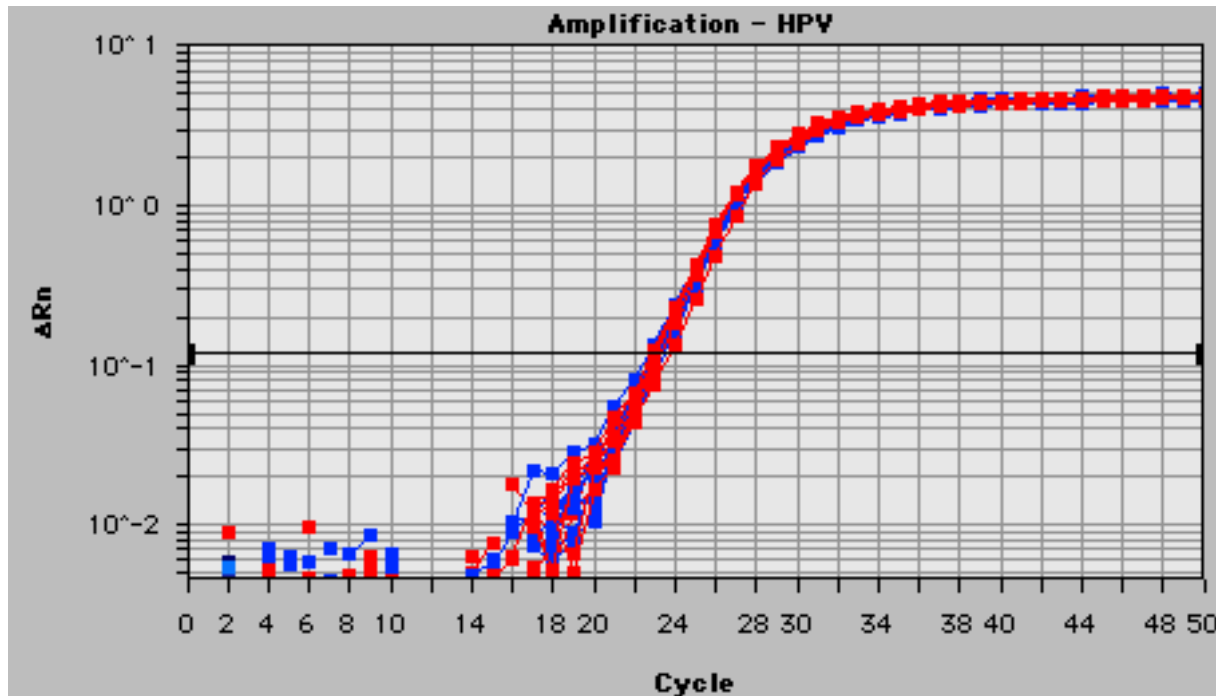


Real time Detection of Serial Dilutions of SiHa cells



Number of cells	Avg Ct	Av Copies/assay
5,000 (red)	27.4	3,100
500 (blue)	31.0	245
50 (lavender)	34.1	29
5 (dark blue)	37.6	3
0.5 (green)	>50	0

Real-time Quantitation: Cell Mixtures



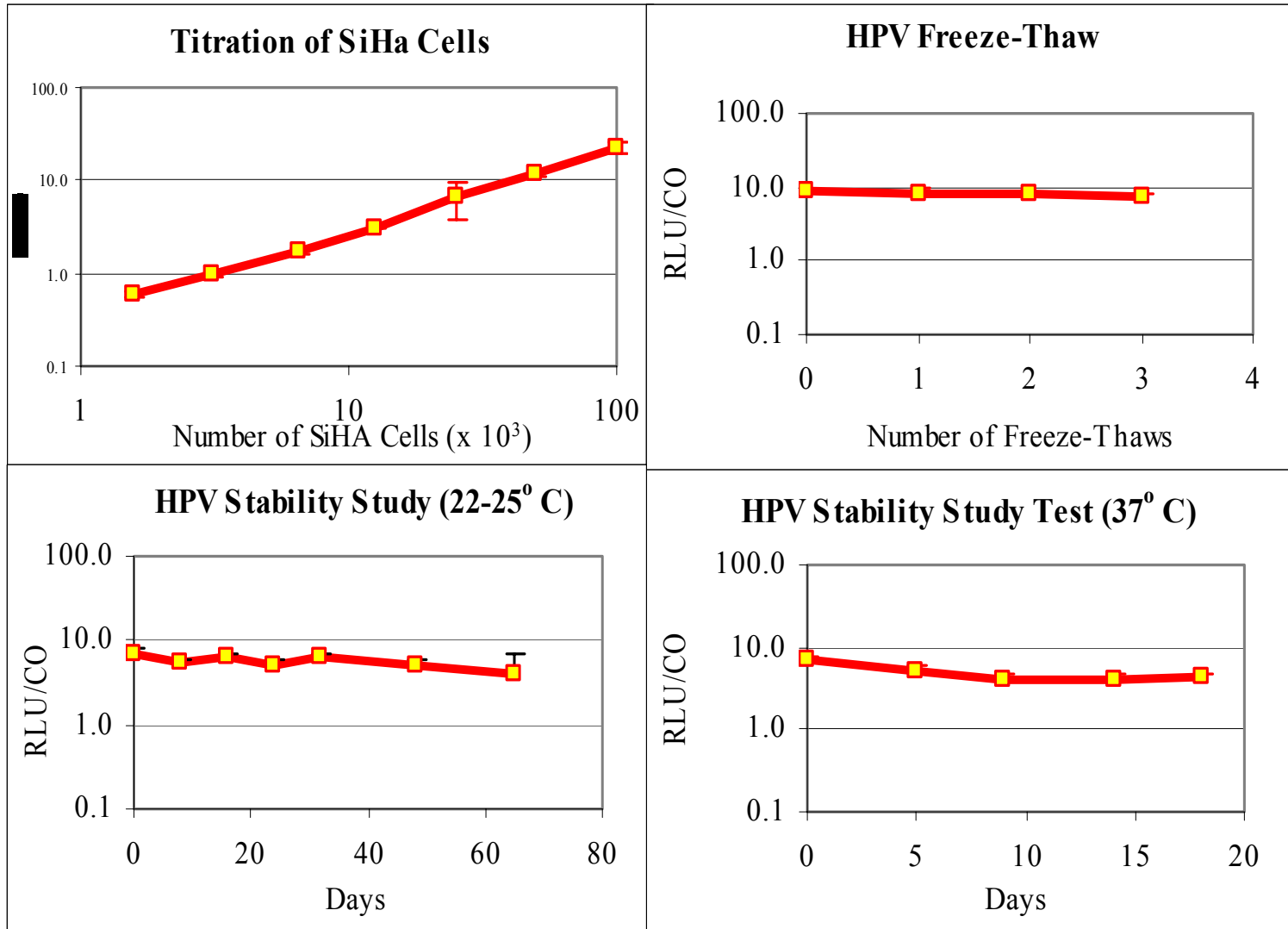
Red: SiHa cells

8,480 copies/ml.

Blue: SiHa & Uninfected cells

8,370 copies/ml.

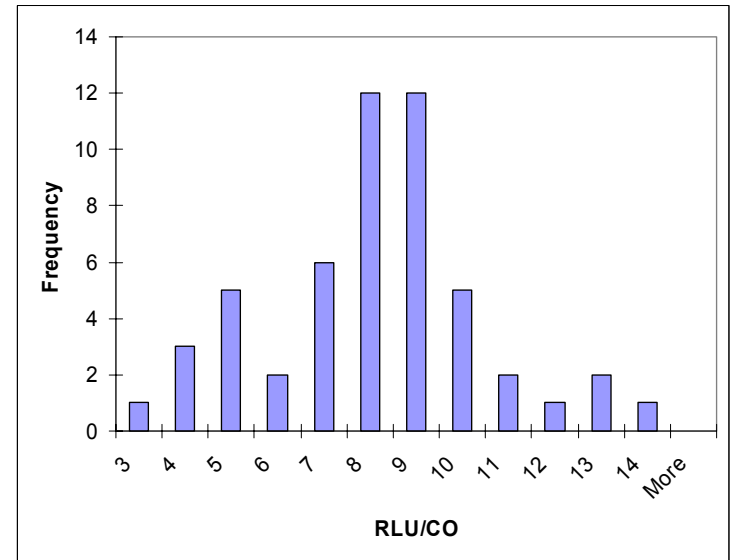
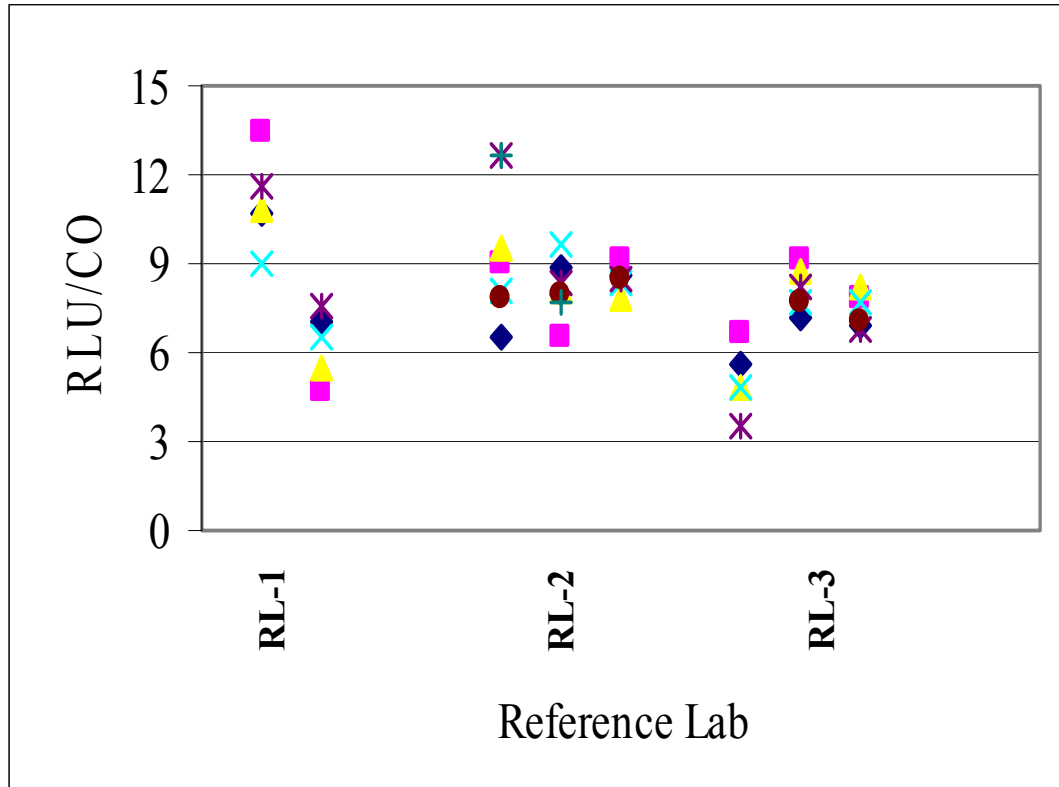
Stability of HPV DNA Controls



Validation Study Design

- Single lot of control material
- 4 testing sites
 - 3 Clinical Labs, 1 IVD manufacturer
- Results reported as RLU/cutoff ratio
- Standard Digene Protocol
- 52 observations
- 8 assay runs
 - 4 assay runs manual equipment
 - 4 assays automated “Rapid Capture”
- 2 kit reagent lots

Field Study Results



N = 52
Mean = 7.7
Median = 7.8
SD = 1.08

HPV DNA Control: Conclusion

- Convenient format compatible with Cytology specimen collection
 - Well accepted by technologists
 - Well developed cell button formation
 - Minimal training for use and interpretation
- Mimics Low HPV Level liquid cytology sample
 - Consistent, well characterized control
 - Controls for entire testing process
- Stable, consistent product
 - Stable up to 65 days at 22-25⁰ C
 - Reflects >2 years of refrigerated storage

HPV ACCURUN Products

In 4 ml PreservCyt

A370 – HPV Positive Control

A371 – HPV Negative Control

In 2 ml SurePath

A870 – HPV Positive Control

A871 – HPV Negative Control

Acknowledgements

BBI Diagnostics

- Alan Doty
- Barbara Weiblen
- Harmesh Sharma

BBI Biotech

- Janet Lathey
- Yiping Zhang
- Anekella Bharathi
- Ron Meixell

Digene

- Iwona Mielznska

Cleveland Clinic

- Susan Schindler

Johns Hopkins Clinical Lab

Quest Diagnostics Lab

SiHa cells are used under a Material License Agreement with the NIH.
Y. Ito Exp. Biol. Med. 135: 543-545, 1970.