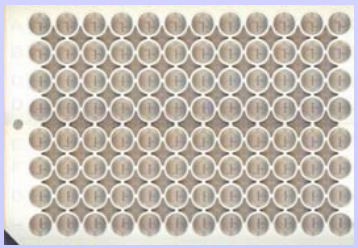
DR. Chip
INTRODUCTION

Bringing Innovative Products and Total Solutions to the Modern Molecular Diagnostics



What is DR. ?

DR. =DNA+RNA

=Doctor

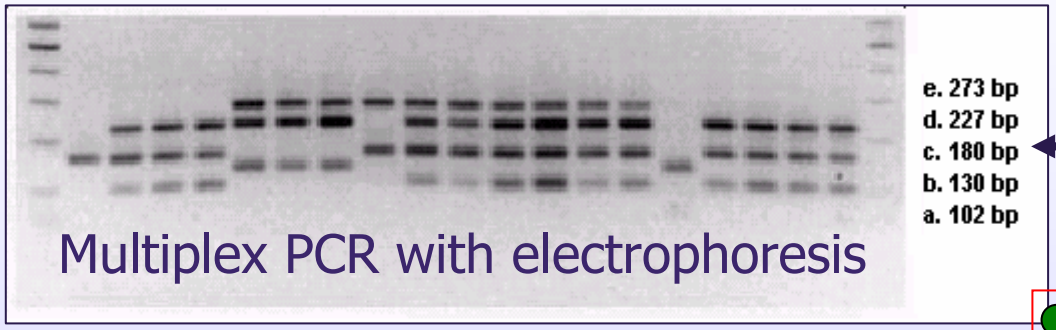
=Expert

DR. Chip aims to use DNA and RNA to give a new tool for diagnosis

The Needs of a Biochip

Biochip vs Multiplex PCR

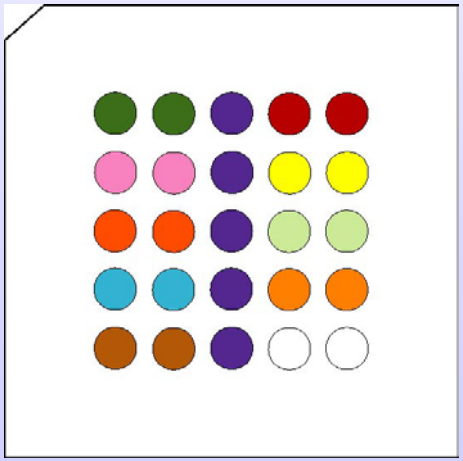
Numerous reasons including following,



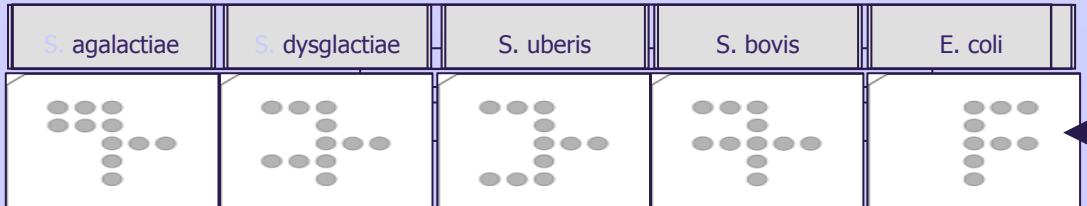
Multiplex PCR with electrophoresis

← Good multiplex PCR and electrophoresis but difficult to identify correct fragments

PCR →



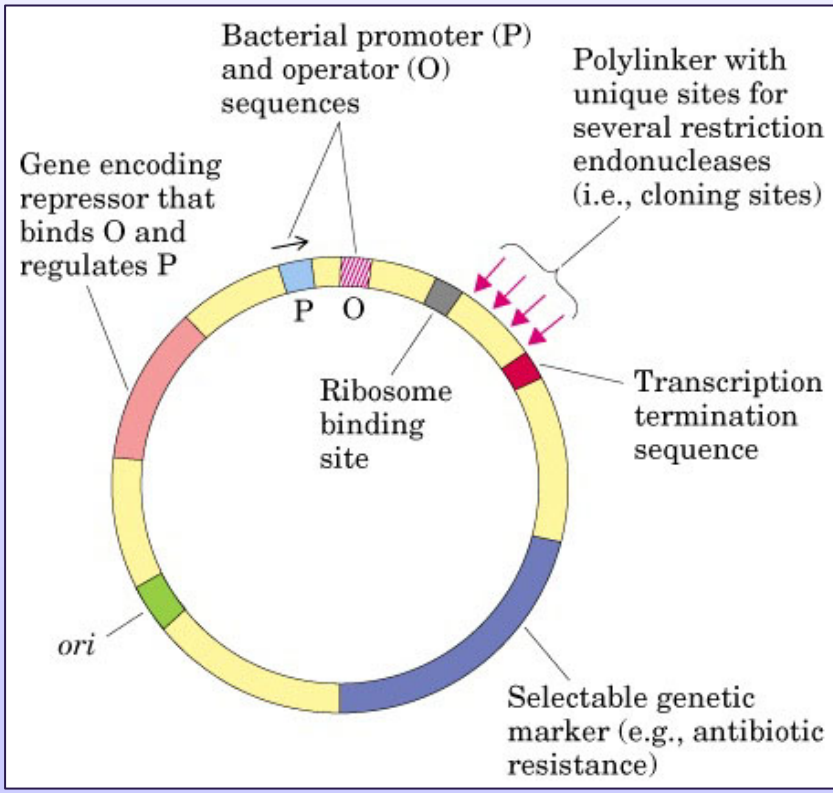
- *Streptococcus* spp.
- *Strep. agalactiae*
- *Strep. bovis*
- *Strep. dysgalactiae*
- *Strep. uberis*
- *E. coli*
- *Staphyl. aureus*
- Positive control
- Negative control
- Blank



← **Easy-recognized pattern with sequence validation**

Design Flow Chart

- Search target genes (Gene bank)
- Primer design, testing, and validation
- Probe design, testing, and validation



Kit contains

- Extraction Reagents*
- Primer Mixture
- DNA Polymerase
- DR. Hyb Buffer
- Blocking Reagent
- Wash Buffer
- Strep-AP
- NBT/BCIP
- Detection Buffer

Products

FOOD Related Products

- DR. Food Chip Series – **Foodborne pathogens**
- DR. Milk Chip – **Mastitis pathogens**
- DR. WIT Chip – **Marine Shrimp Viral Diseases**

Products

Human Related Products

- DR. RV Chip - Respiratory viruses, including SARS
- DR. EV Chip - Enterovirus
- DR. HPV Chip - Human Papillomavirus
- DR. RB Chip - Respiratory bacteria
- DR. TB Chip - Mycobacterium tuberculosis
- DR. HBV Chip - Hepatitis Type B & Lamivudine drug resistant mutations.

Coming up…

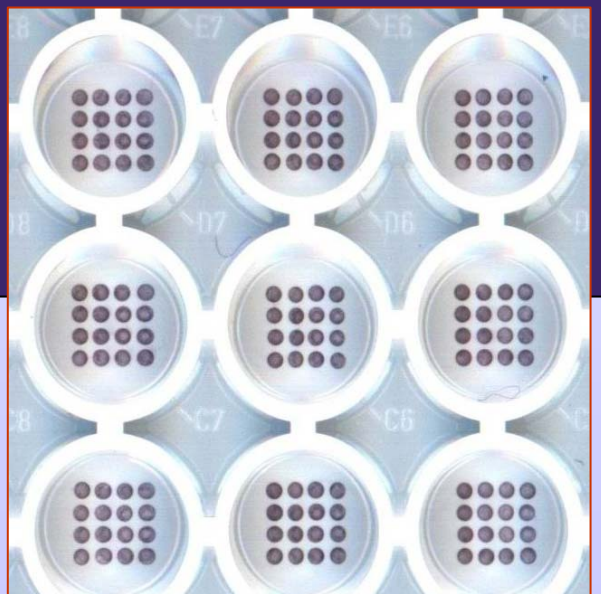
DR. Livestock DNA Chip

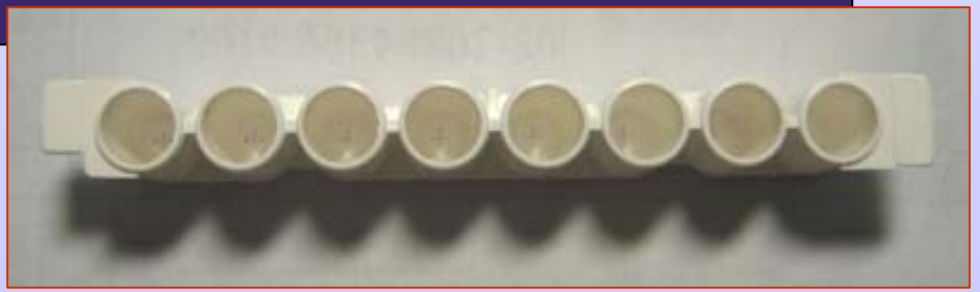
DR. Orchid virus Chip

DR. Porcine Chip

platform

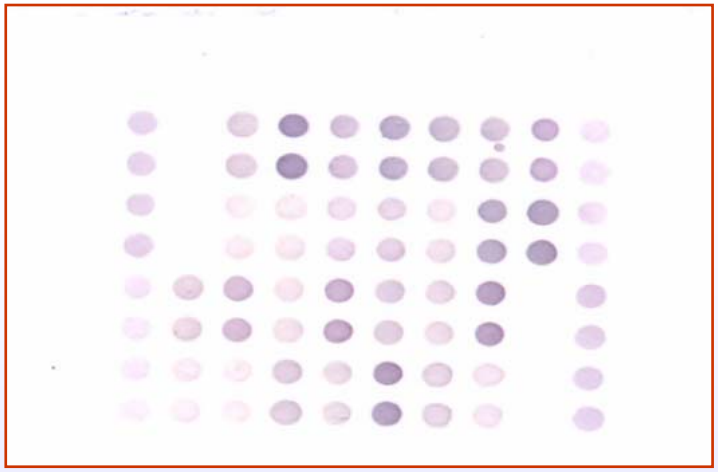
- Polychip
- New 3 well
- C8 strips
- 96 well





Platform Characteristic

- Low noise
- Easy to use
- Cost saving



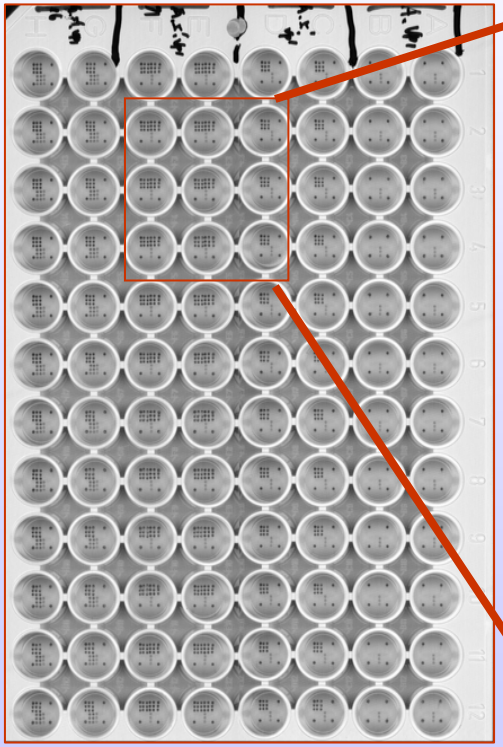
DR Chip polymer chip (low noise

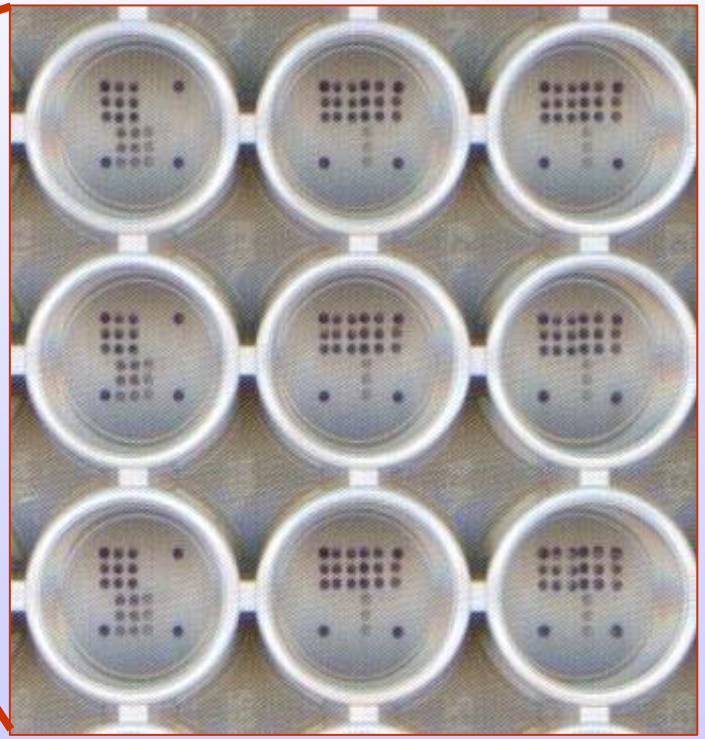
DR. AiM™ Technology
Arrays-in-Microplate

High Throughput Screening Array
Array Reader and
Automatic Analysis System

DR. AiM™
Microplate-based Diagnostic Array

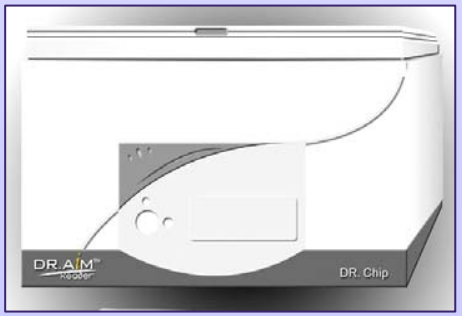
- DR. AiM™ - standard 96-well plate, which is pre-spotted with specific probes at the bottom of the well to capture gene(s) of interest.





Key Components

- Microarray Plate
- Mini Oven
- Fluidic Station
- Biochip Reader
- Biochip Analysis Software



LifeLineLab

Semi-automatic system

DNA Extraction

PCR

Hybridization oven

Color developing

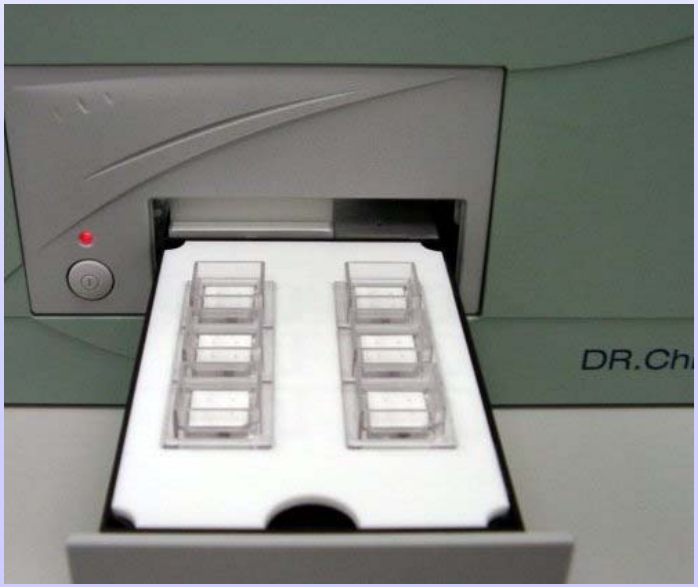
reading

DR. AiM™ Reader

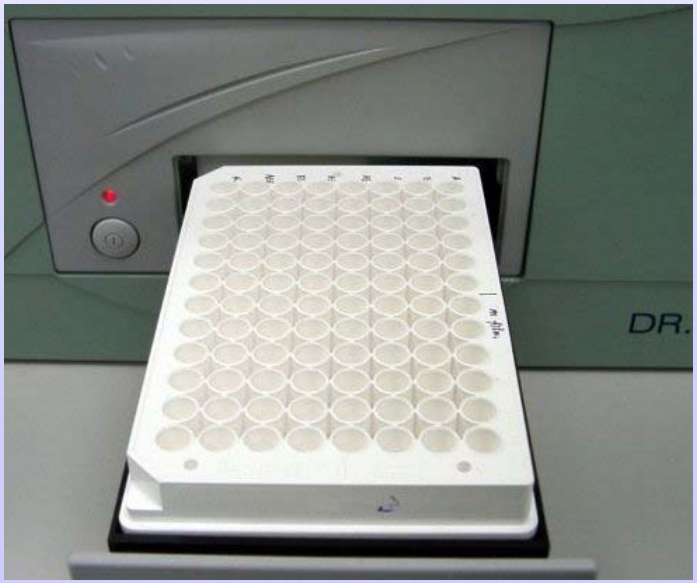
- DR. AiM™ Reader- a sophisticate device that capture image of DR. AiM™

Designed to accommodate
1. standard 96-well microtiter plate
2. DR. PolyChip (3 wells)

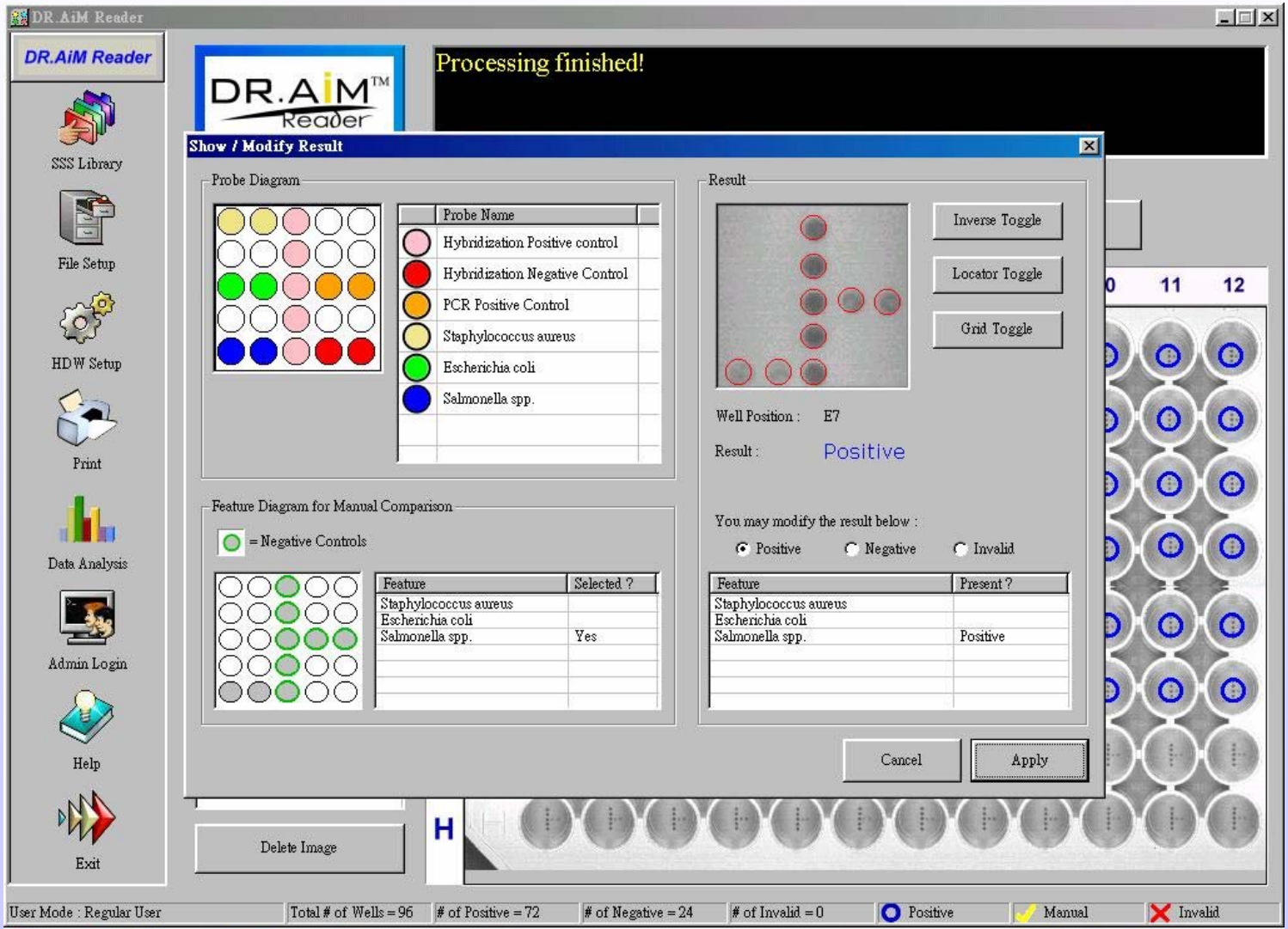
DR. PolyChip (slide format, 3 well / slide)



96-well microtiter

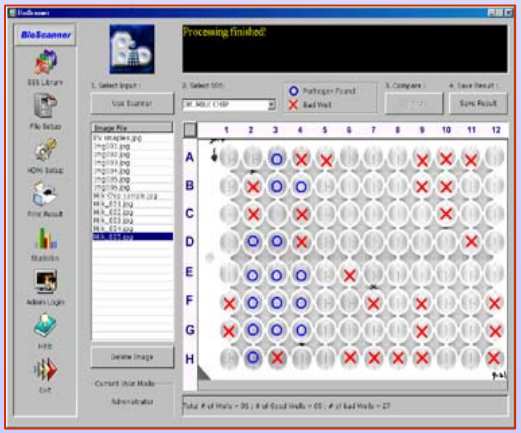


DR. AiMsoft™

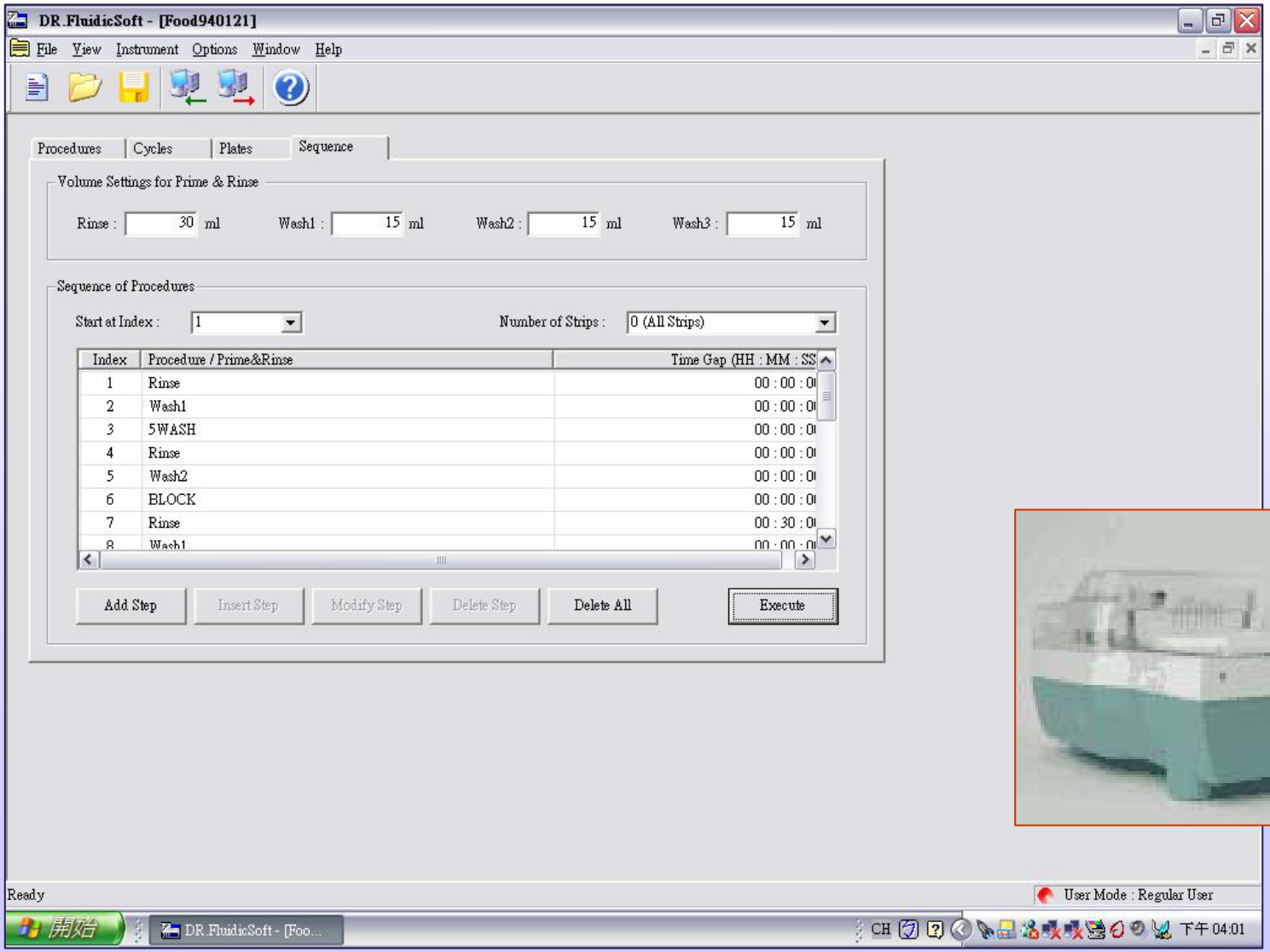

DR.AiM Reader
DR.AiM Reader
SSS Library
File Setup
HDW Setup
Print
Data Analysis
Admin Login
Help
Exit
DR.AiM™ Reader
Processing finished!
Show / Modify Result
Probe Diagram
Probe Name
Hybridization Positive control
Hybridization Negative Control
PCR Positive Control
Staphylococcus aureus
Escherichia coli
Salmonella spp.
Result
Inverse Toggle
Locator Toggle
Grid Toggle
Well Position : E7
Result : Positive
Feature Diagram for Manual Comparison
= Negative Controls
Feature
Selected ?
Staphylococcus aureus
Escherichia coli
Salmonella spp.
Yes
You may modify the result below :
Positive
Negative
Invalid
Feature
Present ?
Staphylococcus aureus
Escherichia coli
Salmonella spp.
Positive
Cancel
Apply
Delete Image
User Mode : Regular User
Total # of Wells = 96
# of Positive = 72
# of Negative = 24
# of Invalid = 0
Positive
Manual
Invalid

BioChip Analysis Software

- User-friendly programmable interface
- Imaging and Analysis
  - Background noise reduction
  - Template Library Directory Setup
  - Date Storage and Output in Excel
  - Default Setup



DR. Fluidic soft™



Fluidic Station

- A semi-automatic fluidic handling system
  - Eliminate human error
- User-friendly programmable interface
- For 96-well and 8 strips microplate
- Compact in size
  - 6.2 kg
  - 28*22*42 (cm)

Ehance Conjugation / Hybridization efficienc
ELISA / Microarry

Mini Oven

- Accurate Temperature Control
  - Range: Room Temp. ~ 85℃
- Vibration to enhance Hybridization
  - 60 Hz
- Compact in Size
  - 6.2 kg
  - 26*30*17.5 (cm)
- Fast Heat Up

DR. Food™ Kit series

Identify maximum 10 major foodborne pathogens in one test

Staphylococcus aureus, Salmonella spp. E. coli
Yersinia enterocolitica, Bacillus cereus,
Clostridium perfringens, Listeria monocytogenes
Shigella spp., Vibrio spp., Campylobacter

- Application-
  - **Detection of common foodborne bacteria**
  - **Assist with quality control of food processing**
  - **Rapid method for foodborne pathogen disease**

- Process

Food samples→

enrichment overnight →

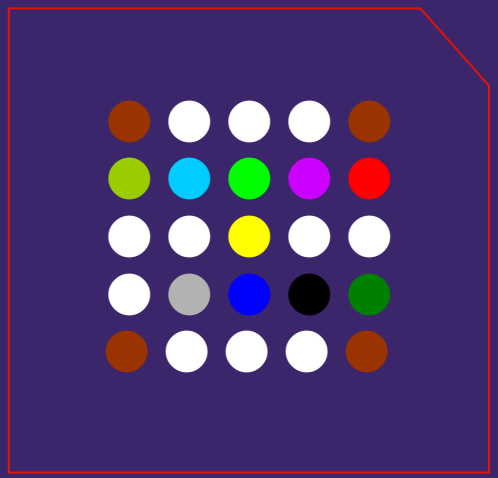
extract DNA→ DNA amplification→

hybridization→ color indication

Kit name	Detecting Bacteria	note
DR. Food – 9 Kit	Staphylococcus aureus, Salmonella spp. E. coli Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, Listeria monocytogenes Shigella spp. Vibrio spp.	Universal pathogens detection
DR. Food –10 Kit	Staphylococcus aureus, Salmonella spp. E. coli Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, Listeria monocytogenes Shigella spp. Vibrio spp. Campylobacter	Universal pathogens detection
DR. Food – S Kit	Staphylococcus aureus, Salmonella spp. E. coli, Listeria monocytogenes Vibrio cholerae.	QC for Sea food material process

DR. Food™ Kit

- Designing and Probe Allocation



	Hybridization Positive Control
	Staphylococcus aureus
	Salmonella spp.
	Escherichia coli - Shigella spp.
	Listeria monocytogenes
	Bacillus cereus
	Yersinia enterocolitica
	Vibrio spp.
	Clostridium perfringens
	PCR positive control
	Negative control

Performance

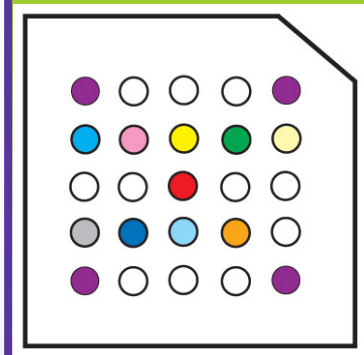
Detection Limit (cfu/mL)

Pathogen	Sensitivity	Pathogen	Sensitivity
Staphylococcus aureus	$10^9 \sim 10^4$	*Listeria monocytogenes*	$10^8 \sim 10^4$
Escherichia coli	$10^8 \sim 10^4$	*Salmonella* spp.	$10^9 \sim 10^4$
Yersinia enterocolitica	$10^8 \sim 10^4$	*Shigella* spp.	$10^8 \sim 10^4$
Bacillus cereus	$10^8 \sim 10^3$	*Vibrio* spp.	$10^9 \sim 10^4$
Clostridium perfringens	$10^8 \sim 10^3$		

Mastitis Factors and Diagnosis

- The most costive cow disease
- According to US National Mastitis Council
  - US$ 184 per cow
  - US$ 184,400 per 100 cow herd
  - US$ 1.7 billion in US
- To detect and identify the pathogens
  - Choosing the right treatment
  - Increasing milk production and milk quality

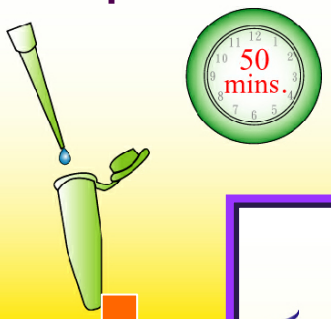
DR. Milk Chip Diagnostic Process



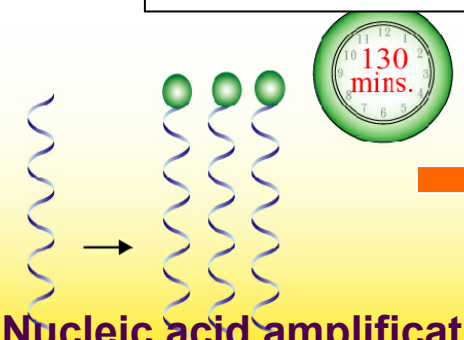
- Hyb Positive Control
- *Streptococcus spp.*
- *Strep. agalactiae*
- *Strep. bovis*
- *Strep. dysgalactiae*
- *Strep. uberis*
- *E. coli*
- *Staphyl. aureus*
- *Mycoplasma bovis*
- PCR Positive Control
- Blank

Sample preparation

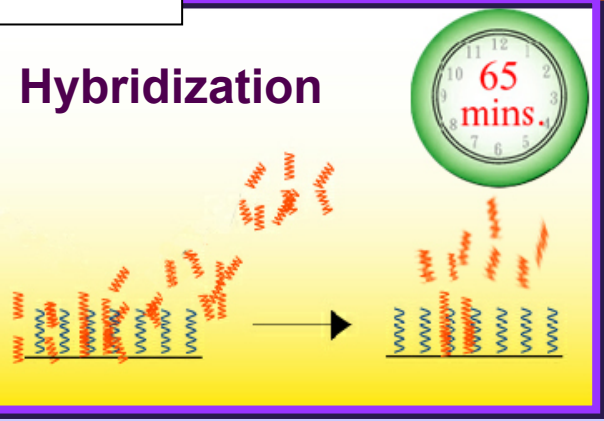
emove fat and ucleic acid purification



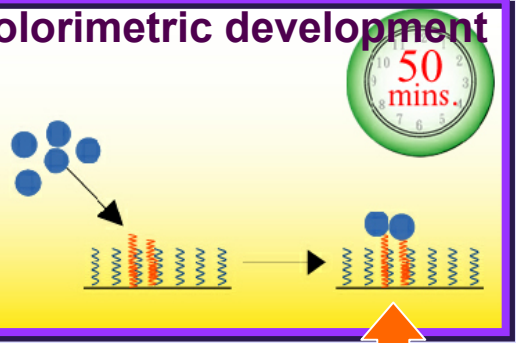
Nucleic acid amplificati n



Hybridization



Colorimetric development



Bacteriology Culture vs. DR. Milk Chip

Bacteria species	Bacteriology culture	DR. Milk Chip
Streptococcus spp.	At least 2 days	Detect 7 mastitis-causing bacteria simultaneously in 6 hours
Strep. uberis	5 days	
Strep. bovis		
Strep. agalactiae		
Strep. dysgalactiae		
Staphyl. aureus	5 days	
E. coli	4 ~ 7 days	
Mycoplasma bovis	7~ 10 days	

Advantages for using DR. Milk Chip：

Parallel Diagnosis：

Detect 7 mastitis-causing bacteria (3 contagious and 4 environmental bacteria) simultaneously

- **Early Diagnosis, Early Treatment：**

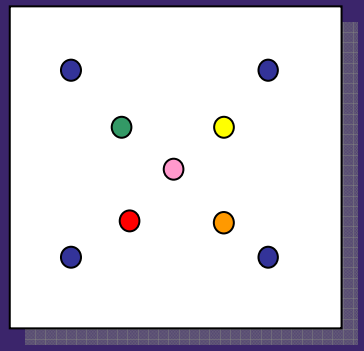
Milk separately, decrease the dairy-processing fee and the cost for treatments, as well as habitual abuse of antibiotics by diagnosing in the early stage.

Minimize the loss by segregation, observation and treatments from detecting in the early stage.

- **Other Advantages：**

Increase in milk production and quality, as well as marketing competitiveness by inspecting periodically

DR. Livestock DNA Chip

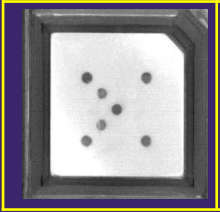
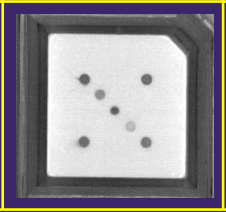
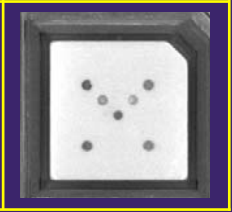


- Porcine-specific probe
- Bovine-specific probe
- Goat/sheep-specific probe
- Livestock conserve probe
- PCR control
- Hybridization control

Application: DR. Livestock™ Kit is a DNA-based system designed for speciation of pork, beef and goat/sheep meat or detection of MBM in feedstuffs

Sensitivity : The detection limit is 0.1% W/W

Process : The entire operation processes require 6 hours

porcine	Bovine	Goat
		

LifeLineLab

Porcine Chip

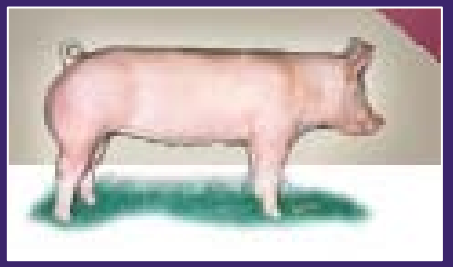
Purpose: To detect most common virus in Swine.

Target :

- **CSFV : Classical Swine Fever Virus**
- **SVDV : Swine Vesicular Disease Virus**
- **BVDV : Vovine Viral Diarrhea Virus**
- **PCV : Porcine Circovirus**

Application : Farm swine health monitoring, infection control.

- **Cooperation with Council of Agriculture**

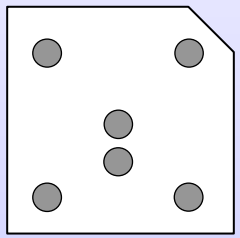


Future Biodiagnostic Chip

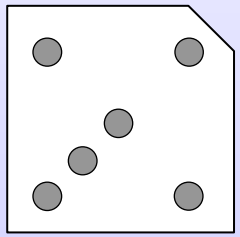
Portable Meter(PDA)

Disposable Chip

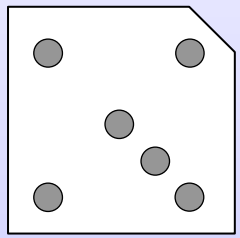
DR. RV™ IVD Kit



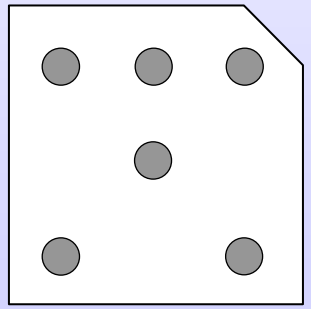
INF A



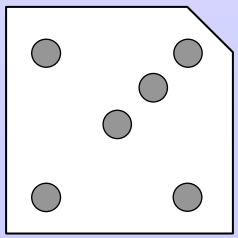
INF B



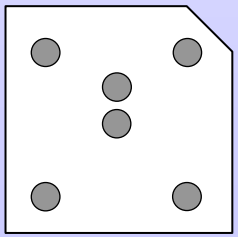
RSV



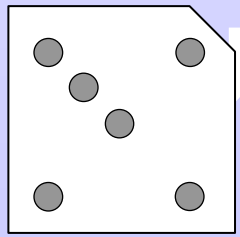
Coronavirus (SARS)



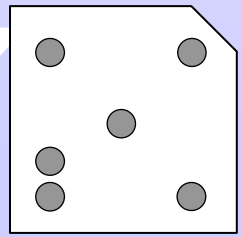
PIV 1



PIV 2



PIV 3



Adenovirus

DR. RB™ IVD Kit

Mycoplasma pneumoniae

Chlamydia pneumoniae

Legionella pneumophila

DR. HPV™ IVD Kit

- Detail clarify the quantity and types of HPV when testing with Pap test
- Keep tracking the growing process of cervical cancer definably
- Detecte many different types of HPV accurately !
- Including many type→

HPV high-risk group: 16,18, 31, 33, 35, 39, 45, 51,52, 56, 58, 59, & 68

HPV low-risk group: 6, 11, 42, 43, & 44

HBV Diagnostic Chip

- Purpose-
  - Detect and identify Hepatitis Type B viruses and genotyping A- F.
  - Detect Lamivudine drug resistant mutations.
- Process-
  - Serum→virus DNA→PCR→hybridization→ color indication
- Application-
  - Long-term monitoring of HBV treatment and drug therapy
- Collaborating Institute-
  - National Taiwan University Medical Center

DR. TB™ IVD Kit

Mycobacterium tuberculosis complex (MTBC)

- *Mycobacterium tuberculosis*
- *Mycobacterium bovis*
- *Mycobacterium bovis BCG*
- *Mycobacterium africanum*
- *Mycobacterium microti*

MOTT or NTM

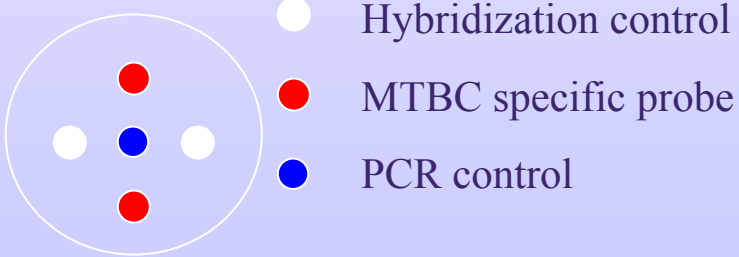
Mycobacterium other than tuberculosis

Non-tuberculosis *mycobacterium*

Mycobacterium tuberculosis complex screening/drug resistant chip

- Fast (Diagnose MTBC infection in 6 hours)
- High sensitivity (Lower than 100 bacteria in specimen can be detected)
- High specificity (Rare false positive result would be detected with specific probes)
- High throughput (Detected in 96 well plate format semi-automatically)
- Cheap

Procedure of MTBC screening/drug resistant chip

MTBC screening chip	MTBC drug resistant chip
DNA extraction from specimen	Culture of MTBC from specimen
PCR amplification of MTBC specific target DNA	DNA extraction from cultured MTBC colony
Chip hybridization test	PCR amplification of MTBC drug resistant related target DNA
	Chip hybridization test

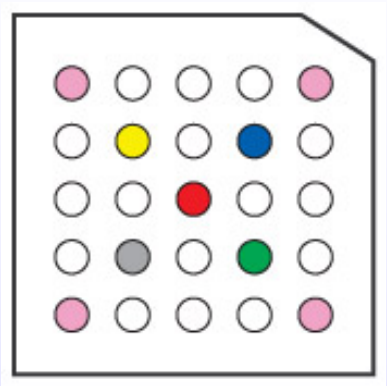
DR.EV™ IVD Kit

- Features:

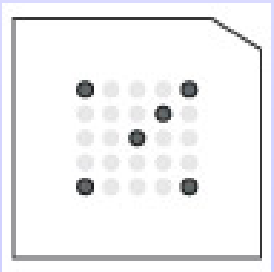
Size: 1 cm X 1 cm

Probes:

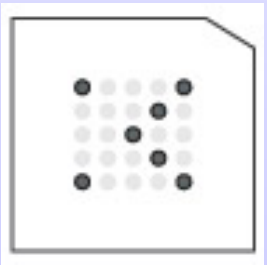
1. Positive & negative controls
2. EV common sequences
3. EV subtype sequences



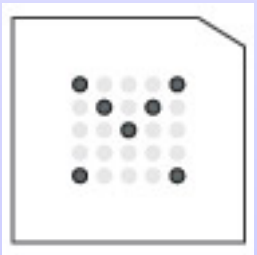
- Hybridization positive contro
- PCR positive control
- EV common sequence
- EV 71
- Cox A16



Enterovirus



EV 71



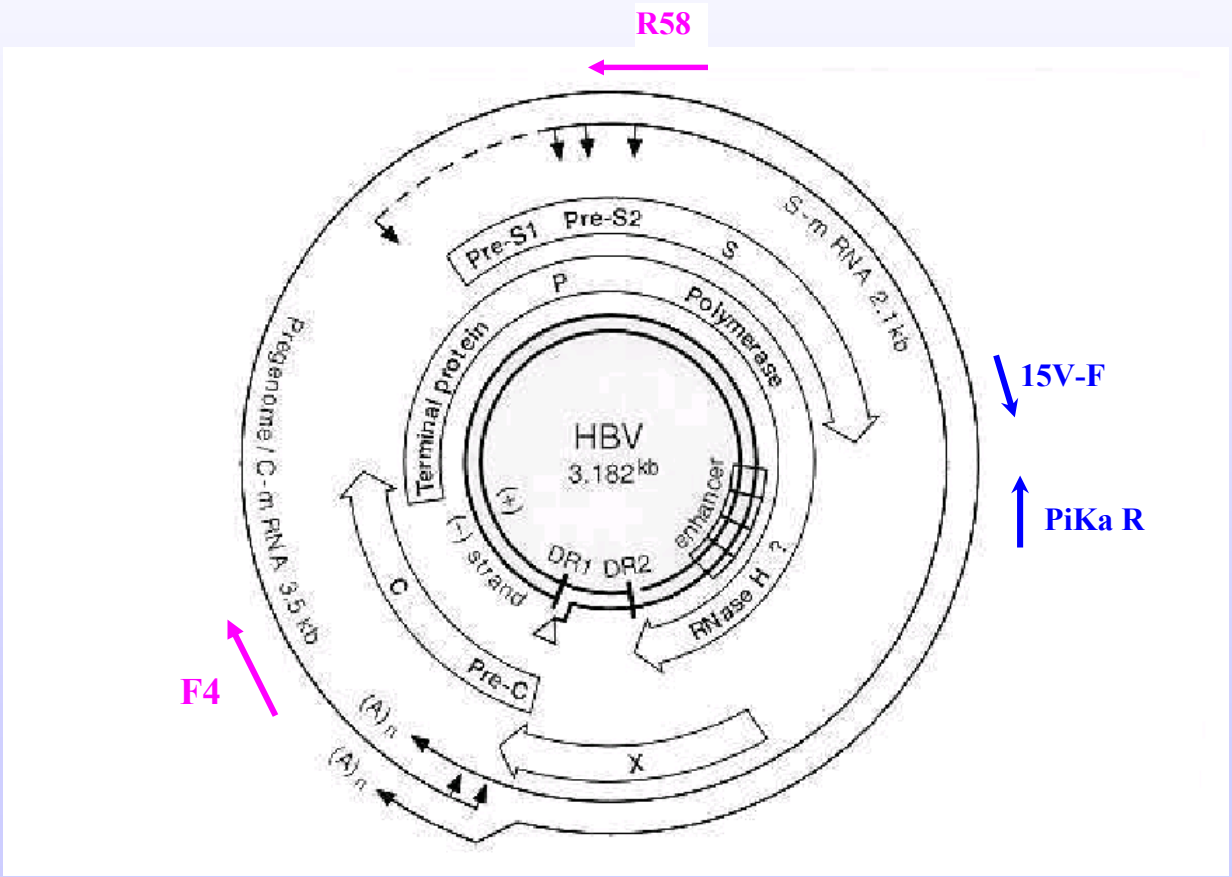
Cox A16

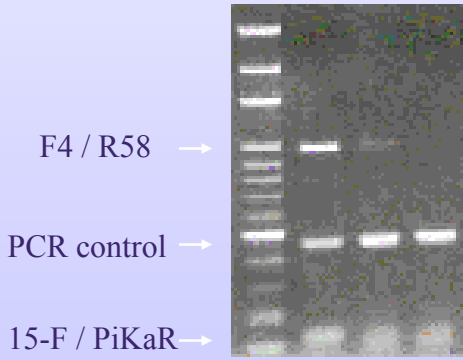
DR. HBV Chip Kit

Introduction:

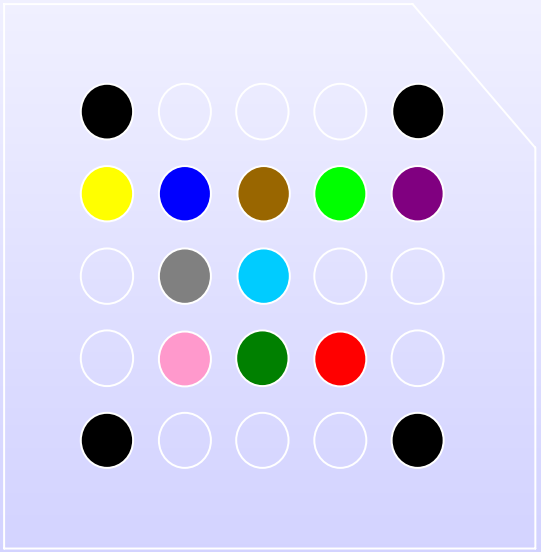
1. Detect and identify Hepatitis Type B viruses and genotyping A- F
2. Detect Lamivudine drug resistant mutations (YVDD, YIDD) during HBV therapy.
3. Sensitivity: 10^5 copies/ml
4. Long-term monitoring of HBV treatment and drug therapy
5. Collaborating with National Taiwan University Medical Center

Design of primers





DR. HBV™ Chip



- Hybridization Positive Control
- Hybridization Negative Control
- PCR Positive Control
- HBV genotype A
- HBV genotype B
- HBV genotype C
- HBV genotype E
- HBV genotype F
- YVDD strain
- HBV common
- YIDD strain

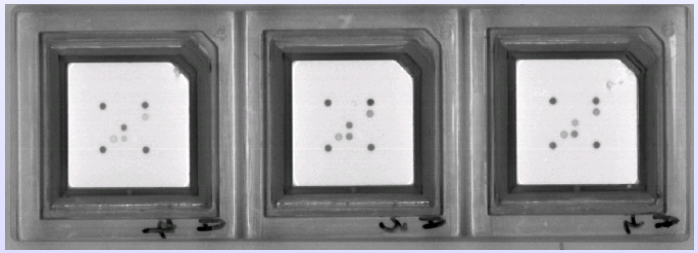
Pattern image examples

HBV genotype A	HBV genotype B	HBV genotype C	HBV genotype D	HBV genotype E
HBV genotype F	HBV genotype B YVDD	HBV genotype B YIDD	HBV Negative	

DR. HBV™ Chip

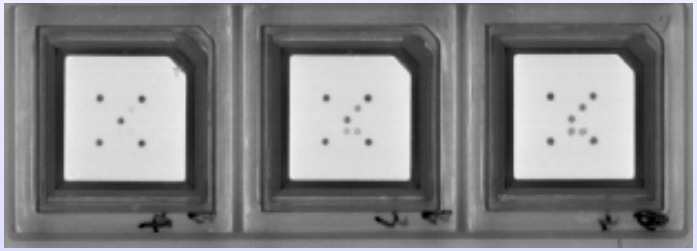
HBV type A (YIDD mutant)

10^5 10^6 10^7 copies / ml



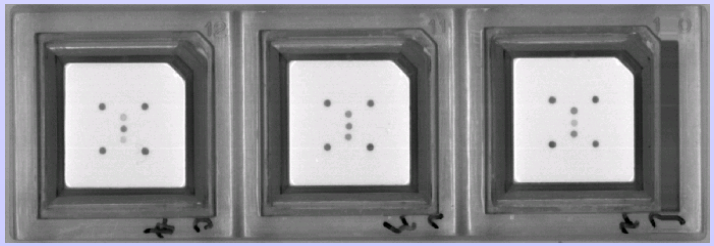
HBV type B (YVDD mutant)

10^5 10^6 10^7 copies / ml



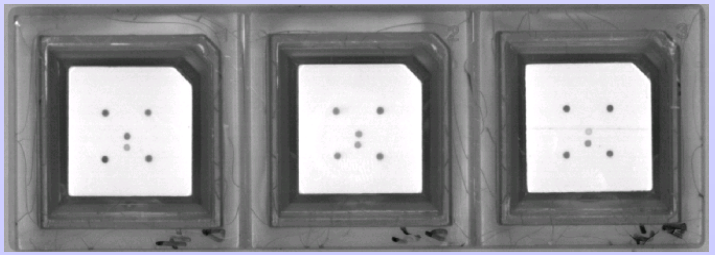
HBV type C (non-mutated)

10^5 10^6 10^7 copies / ml



HBV type D (non-mutated)

10^5 10^6 10^7 copies / ml



Assay protocol:

HBV DNA extraction from patient'serum (200 µl)
QIAamp Blood Mini kit

DNA amplification (PCR method)

Hybridization with chip

Detection with DR.AiM reader

RFLP vs DR. HBV chip

No.	RFLP	DR HBV chip	No.	RFLP	DR HBV chip
R1	B	B	R31	B	B
R2	B	B	R32	B	B
R3	C	C	R33	B	B
R4	B	B	R34	B	B
R5	C	C	R35	C	C
R6	C	C	R36	B	B
R7	B	B	R37	C	C
R8	B	B	R38	B	B
R9	B	B	R39	B	B
R10	C	C	R40	C	C
R11	B	B	R41	B	B
R12	B	B	R42	C	C
R13	B	C	**R43**	B	B
R14	B	B	R44	B	B
R15	B	B	R45	B	B
R16	B	B	R46	B	B
R17	B	B	R47	B	B
R18	B	B	R48	B	B
R19	B	B	R49	B	B
R20	B	B	R50	B	B
R21	C	C	R51	B	B
R22	B	B	R52	C	C
R23	C	C	R53	C	C
R24	B	B	R54	C	B
R25	C	C	R55	B	B
R26	B	B	R56	C	C
R27	B	B	R57	B	B
R28	C	C	R58	C	C
R29	B	B	R59	B	B
R30	C	C	R60	B	B

RFLP method is from "FEBS Letter" 1999", volume 450, Issue1-2, P66-71. "HBV genotype assignment

DNA Sequencing vs DR. HBV Chip

No.	DNA Sequencing	DR. HBV chip		No.	DNA Sequencing	DR. HBV chip
D1	YIDD	YIDD		D22	YIDD	YIDD
D2	YVDD	YVDD		D23	YVDD	YVDD
D3	YIDD	YIDD		D24	YIDD	YIDD
D4	YVDD	YVDD		D25	YVDD	YVDD
D5	YVDD	YVDD		D26	YIDD	YIDD
D6	YMDD	YIDD		D27	YMDD	YMDD
D7	YMDD	YIDD		D28	YVDD	YVDD
D8	YIDD	YIDD		D29	YIDD	YMDD
D9	YMDD	YMDD		D30	YMDD	YMDD
D10	YMDD	YMDD		D31	YIDD	YIDD
D11	YMDD	YMDD		D32	YMDD	YMDD
D12	YMDD	YMDD		D33	YMDD	YMDD
D13	YMDD	YMDD		D34	YIDD	YMDD
D14	YVDD	YVDD		D35	YIDD	YMDD
D15	YVDD	YVDD		D36	YMDD	YMDD
D16	YIDD	YIDD		D37	YVDD	YVDD
D17	YVDD	YVDD		D38	YVDD	YVDD
D18	YVDD	YMDD			**TOTAL**	**84%(32/38)**
D19	YVDD	YVDD			**YMDD**	**83% (10/12)**
D20	YIDD	YIDD			**YVDD**	92%(12/13)
D21	YIDD	YIDD			**YIDD**	77%(10/13)

HBV+ Serum Testing

No	HBeAg	DR HBV chip	Q-PCR (copies/mL)	No	HBeAg	DR HBV chip	Q-PCR (copies/mL)
01	− (0.012)	−	N/A	24	− (0.034)	−	N/A
02	+ (2.697)	+ (B, YMDD)	7,185,000	25	− (0.022)	−	N/A
03	− (0.012)	−	N/A	26	− (0.011)	−	N/A
04	− (0.012)	−	N/A	27	− (0.016)	−	N/A
05	+ (2.598)	+ (B, YMDD)	158,100,000	28	+ (0.196)	+ (B, YMDD)	123,300
06	− (0.022)	−	N/A	29	− (0.015)	−	N/A
07	+ (2.952)	+ (B, YMDD)	302,300,000	30	− (0.045)	−	N/A
08	+ (2.869)	+ (B,YMDD)	19,350,000	31	+ (2.914)	+ (B, YMDD)	3,801,000
09	− (0.013)	−	N/A	32	− (0.020)	−	N/A
10	+ (2.620)	+ (B, YMDD)	232,600,000	33	− (0.018)	−	N/A
11	− (0.014)	−	N/A	34	− (0.013)	−	N/A
12	+ (0.302)	−	N/A	35	+ (2.572)	+ (C,YMDD)	400,700,000
13	− (0.013)	−	N/A	36	− (0.022)	−	N/A
14	− (0.014)	−	N/A	37	− (0.018)	−	N/A
15	− (0.033)	−	N/A	38	− (0.019)	−	N/A
16	+ (2.965)	+ (B, YMDD)	5,071,000	39	− (0.013)	−	N/A
17	− (0.011)	−	N/A	40	− (0.018)	−	N/A
18	− (0.013)	−	N/A	41	− (0.030)	−	N/A
19	− (0.012)	−	N/A	42	+ (2.981)	+ (B, YMDD)	124,600,000
20	− (0.008)	−	N/A	43	− (0.046)	−	N/A
21	− (0.032)	−	N/A	44	− (0.029)	−	N/A
22	− (0.014)	−	N/A	45	+ (3.096)	+ (C,YMDD)	142,000,000
23	− (0.014)	−	N/A				

DR. HPV™ Chip Kit

- Detail clarify the quantity and types of HPV when testing with Pap test
- Keep tracking the growing process of cervical cancer definably
- Detect different types of HPV accurately !

CERVICAL CANCER and HPV

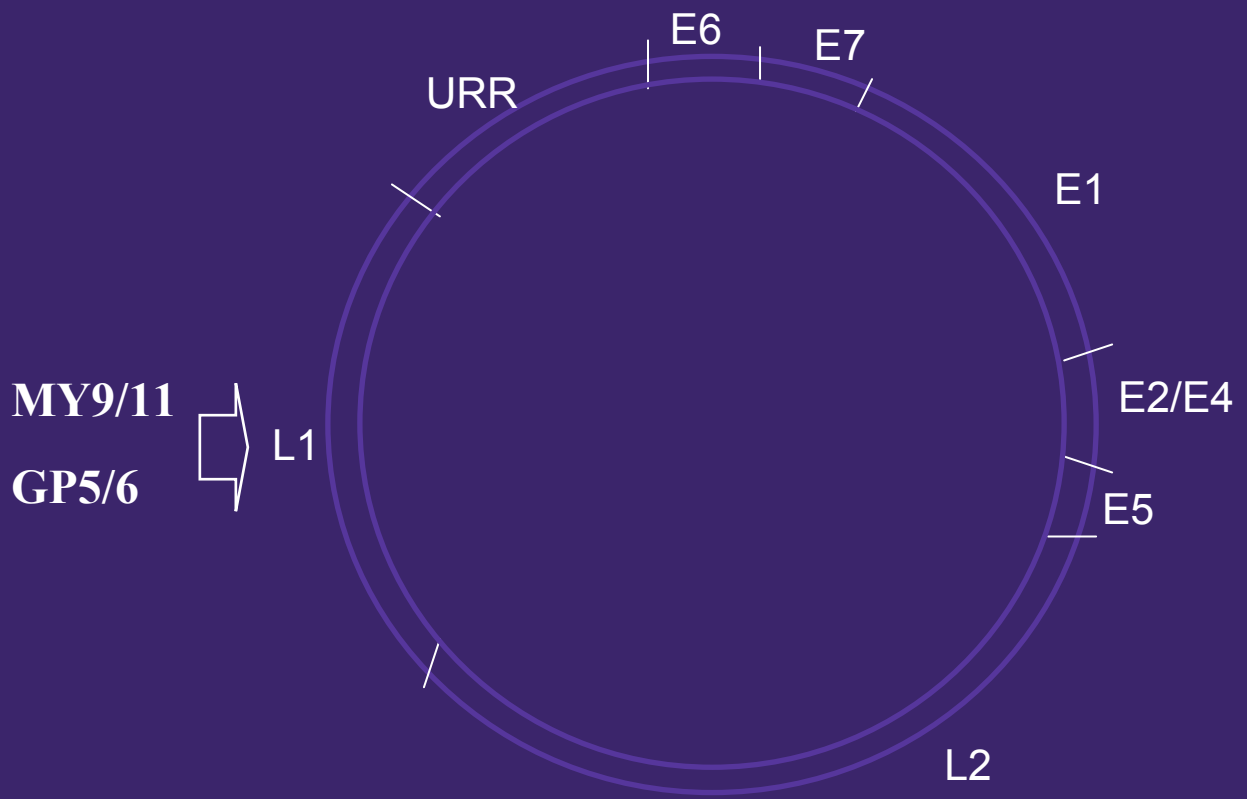
- Widely accepted that human papillomavirus, a sexually transmitted virus, is the major precipitant in the evolution from squamous metaplasia to dysplasia in the transition zone of the cervix

- HPV is the necessary but insufficient cause of cervical cancer

• HPV genomes are found in 99% of premalignant and malignant squamous cell cancer cases worldwide (Walboomers, 2001)

• Presence of high risk HPV (HR-HPV) types (16,18,31,33,34,35,39,45,51,52,56,58,59,66,68,70) are generally recognized as the major risk factor for development of cervical cancer

HPV genotyping



Detect 22 HPV subtypes

High risk :

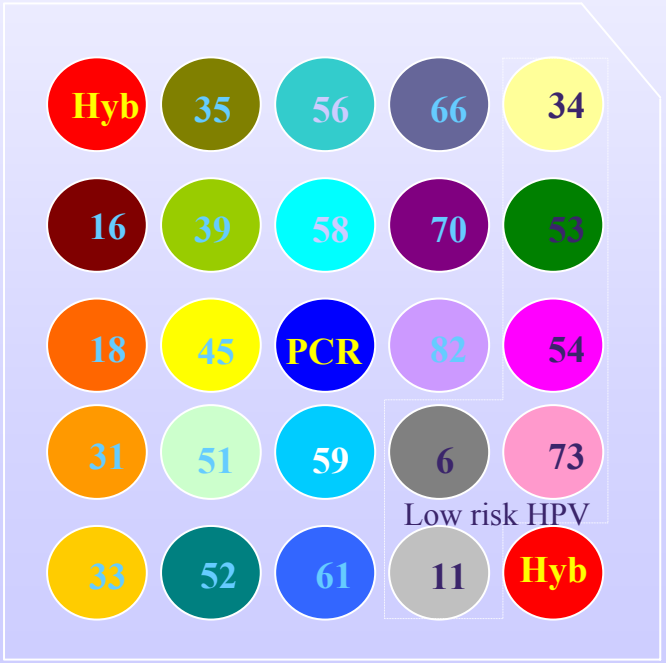
16,18,31,33,35,39,45,51,52,56,58,59,61,66,70,82

Low risk :

6,11,34,53,54,73

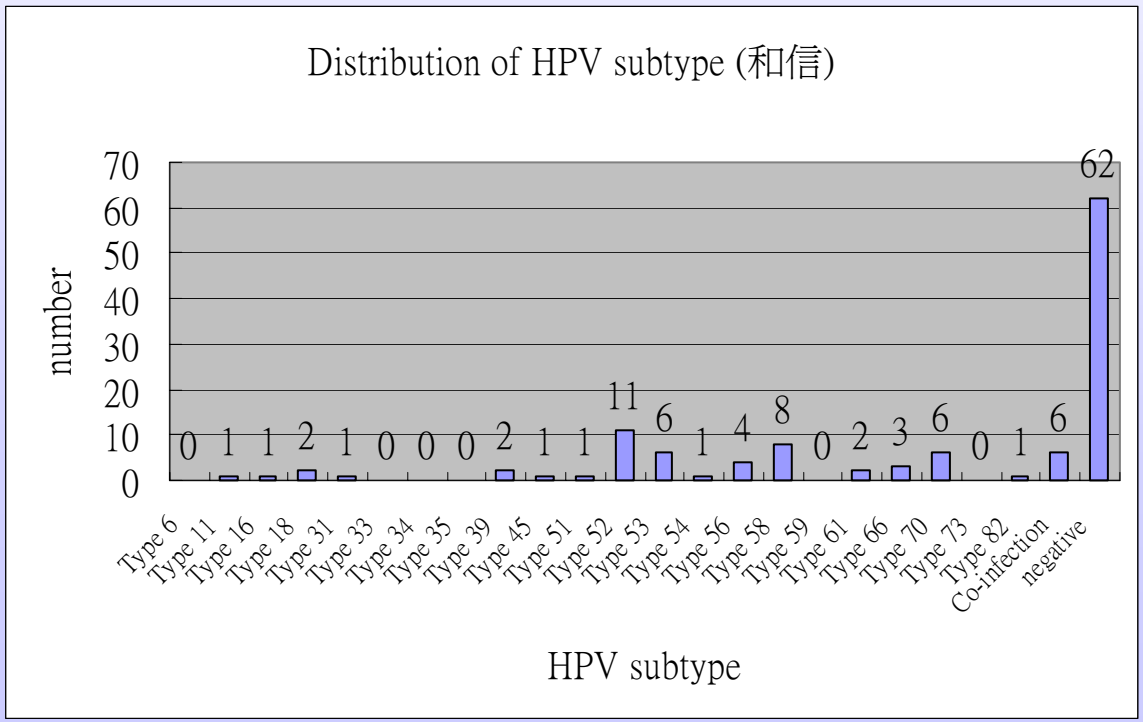
Sensitivity

~10^3 copies based on HeLa cell testing



Testing of HPV chip (I)

- Collaborating with KOO FOUNDATION SUN YAT-SEN CANCER CENTER
- Specimens from Low SIL or ASCUS patients



			ASCUS/L-SIL
Dr.HPV CHIP	Positive	well typing	51
		co-infectio	6
	Negative		62
Positive rate%			48

Prevalence

In cervical lesions

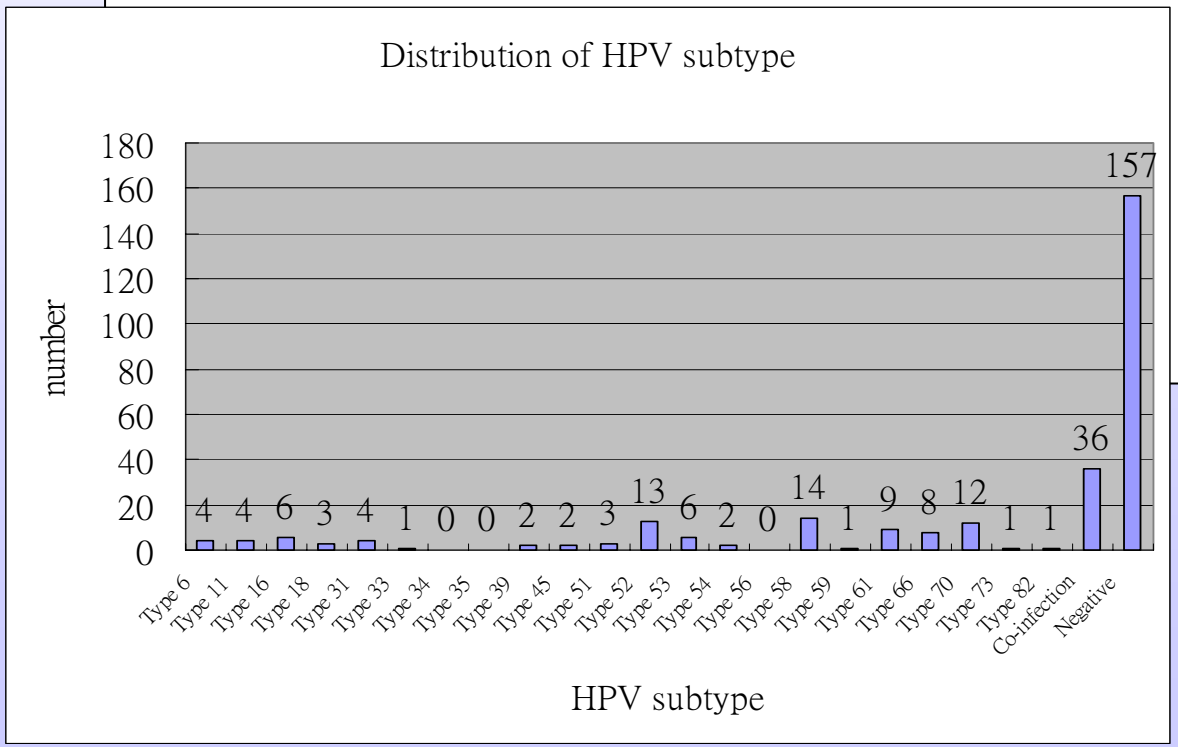
- Squamous carcinoma: 95% association
- HSIL/CIN II, III: 75 -95%
- LSIL/CINI: 60%
- ASCUS: 30%
- Adenocarcinoma: 60% association

(ref: Cuzick et al, 1992; Schiffman et al, 1993; IARC, 1995; Olsen et al, 1995)

HUG
Hôpitaux Universitaires de Genève

Testing of HPV chip (II)

- Collaborating with a local reference laboratory center
- Random collection of swab samples



Dr.HPV CHIP	Positive	Well typing	96
		Co-infection	36
	Negative		157
Positive rate（%）			45.7

HPV high/low risk chip

H/L system: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 61, 66, 70, 82