




Diagnostics

Food Testing

Our Commitment to Food Safety



José Aguirre
Roche Diagnostics
Central America and Caribbean

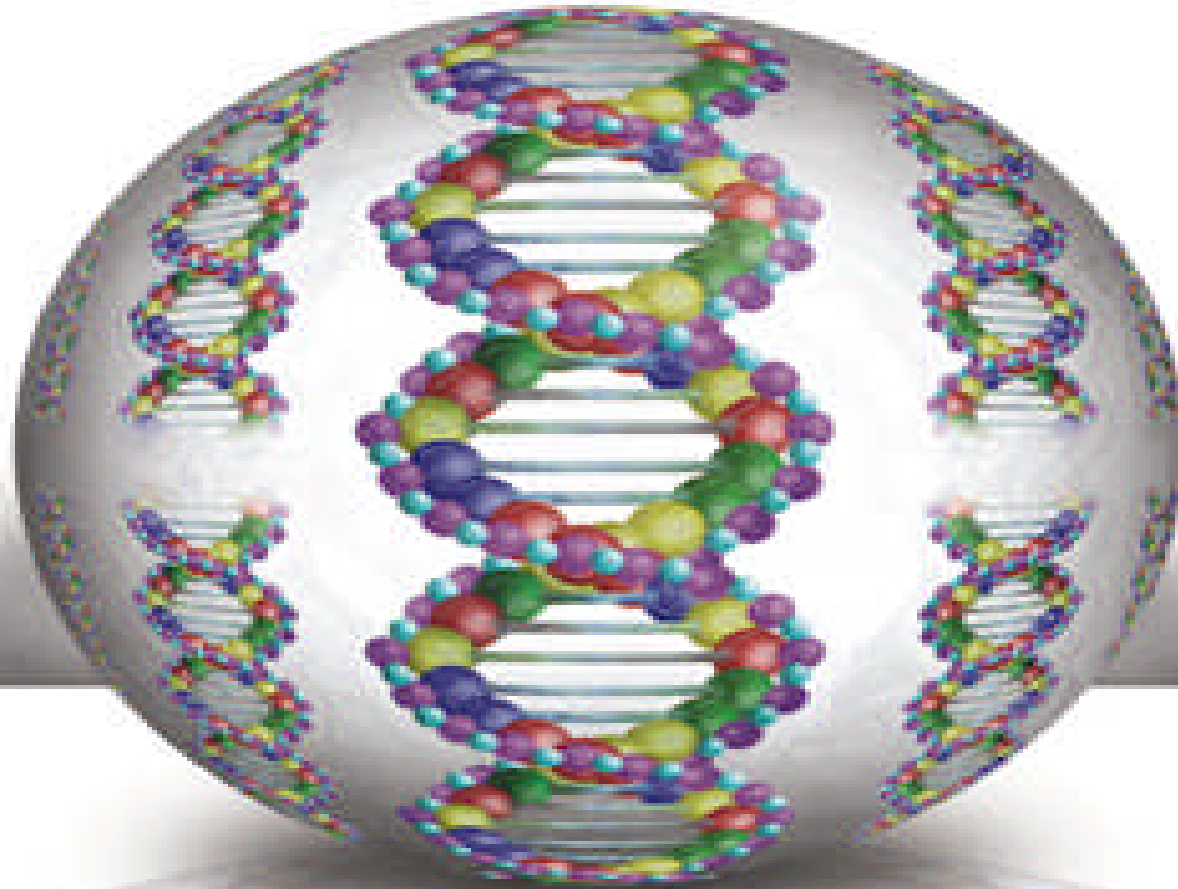
Trinidad, April 5th 2006

Food Testing Integrated Solutions

Content

- Real-Time PCR
- LightCycler[®] 1.5 and 2.0 Instruments
- Kits for Food Testing
 - Foodborne pathogen testing
 - Avian Influenza
- Laboratory Set Up
- Summary

Polymerase Chain Reaction

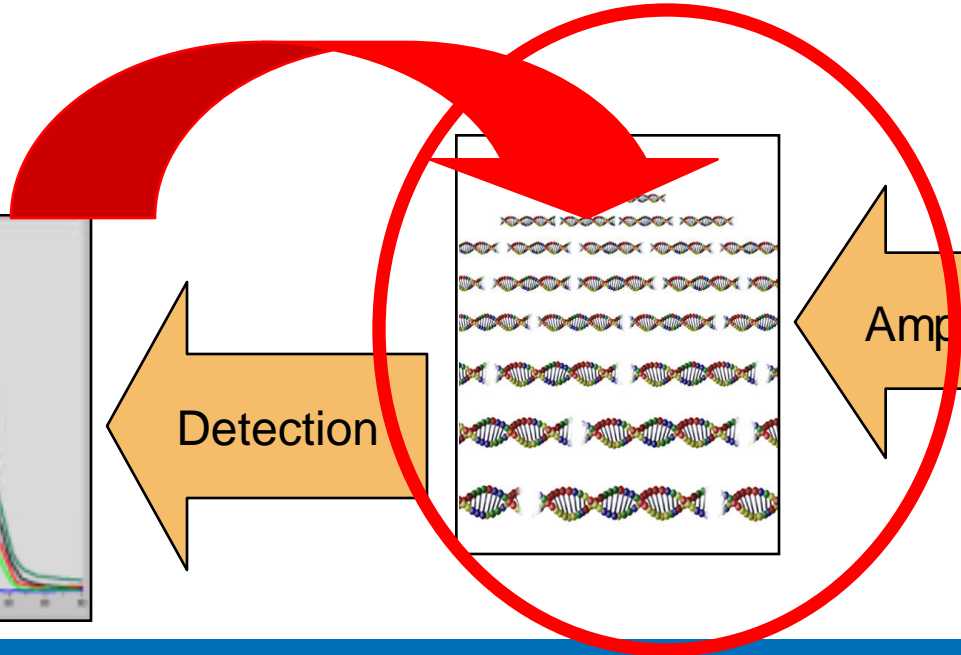
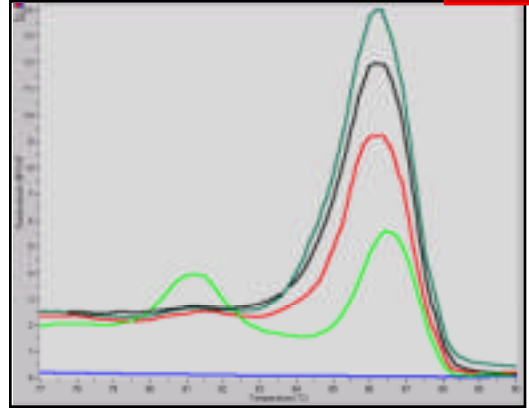
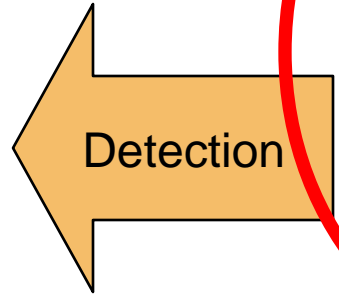
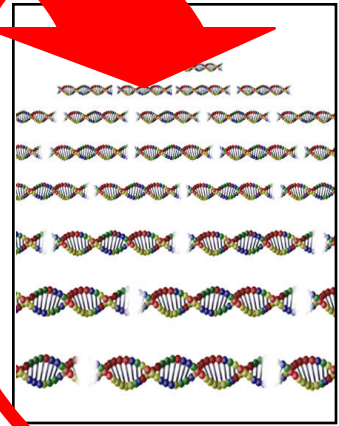
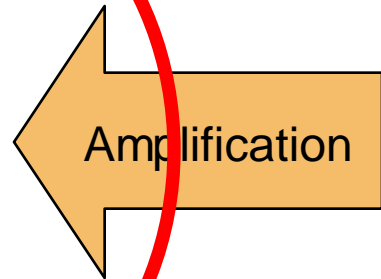
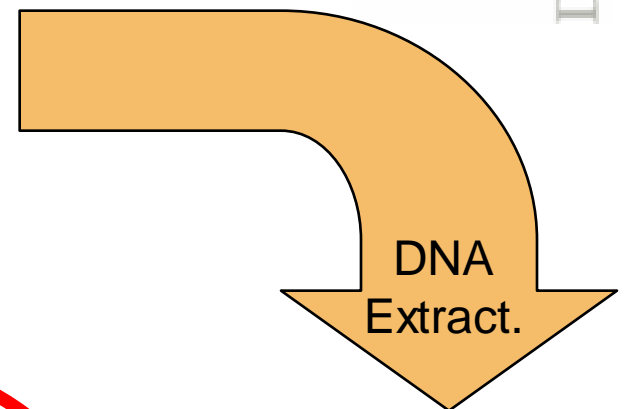
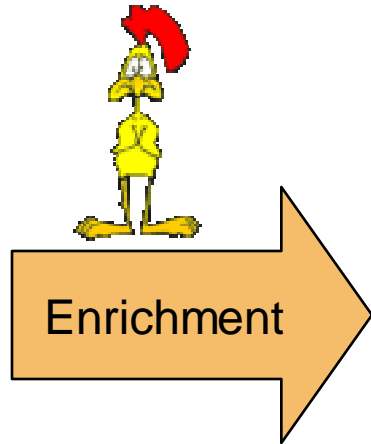


P C R

Real-Time PCR ?



Diagnostics





LightCycler[®] System Technology

The combination of using **ambient air for heating and cooling** of the reaction chamber and specially designed **capillaries with optimized volume to surface ratio** allow one PCR cycle per approximate 30 seconds and a complete PCR run of **35 cycles with 32 capillaries in approximately 30 min.**

The amplification of PCR product is monitored simultaneously in real-time and online with fluorescence dyes.

LightCycler® 1.5 /2.0 Systems *Instruments*



LightCycler® 1.5 Instrument
Proven performance that anyone can afford.

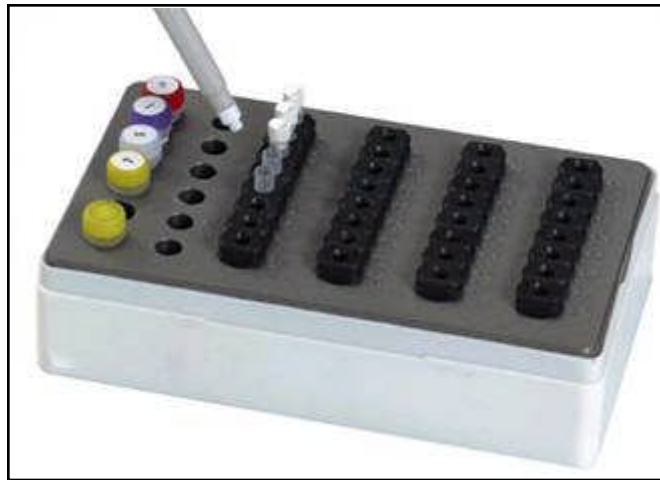


LightCycler® 2.0 Instrument
Enhanced performance that meets the needs of even the most demanding user. Now conforms to European directive for *in vitro* diagnostic medical devices 98/79/EC.

LightCycler[®] 1.5 /2.0 System Components *Additional Products*



**LightCycler[®]
Capillaries**
100 µl and 20 µl




LightCycler[®] Centrifuge Adapters
in a cooling block
LightCycler[®] Capping Tool

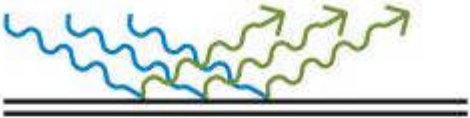


LightCycler[®] Sample Carousels
for 100 µl and 20 µl capillaries


LightCycler[®] Assay Formats



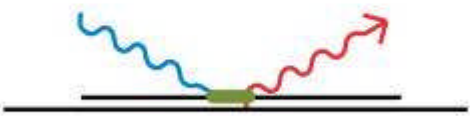
LightCycler 2.0



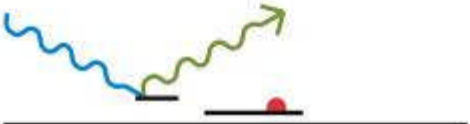
SYBR Green I



SimpleProbe Probes



HybProbe Probes

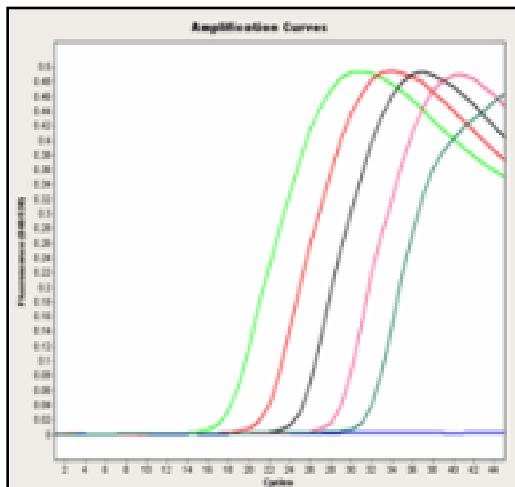


**Hydrolysis Probes
and Universal
ProbeLibrary
Probes**

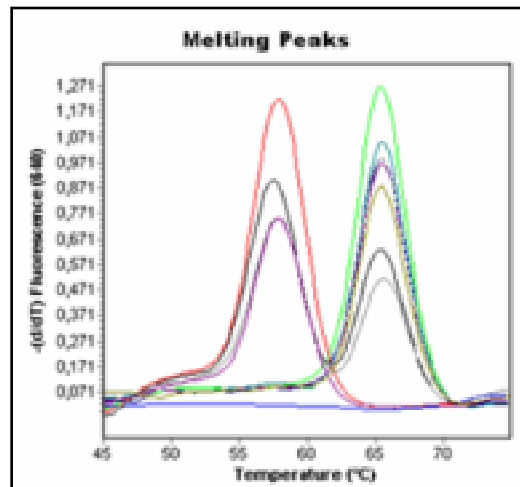
Real-Time PCR

Application principles

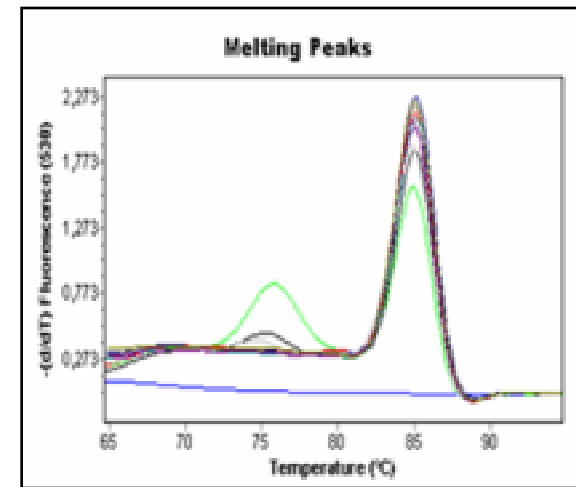
Quantification and qualitative detection



Mutation detection



Product identification





Foodborne Pathogen Testing *Workflow*

Sampling enrichment



**Isolation and purification
of bacterial DNA**



**Amplification and
detection**

25 g homogenized food,
incubated in 225 ml
standard medium
according to BAM
official methods (24-48
h).

**Roche Diagnostics offers an Integrated Solution for rapid
testing of the major foodborne pathogens (within ≤ 2.5 h).**



Foodborne Pathogen Testing *Kits for food testing*

Roche Applied Science offers an **Integrated Solution** for rapid testing of the major foodborne pathogens

➤ DNA Purification Kits

Manual:

High Pure foodproof I Kit
High Pure foodproof II Kit
ShortPrep foodproof I Kit
ShortPrep foodproof II Kit

➤ LightCycler® foodproof Detection Kits

LightCycler® foodproof *Salmonella* Detection Kit
LightCycler® foodproof *Listeria monocytogenes* Detection Kit
LightCycler® foodproof *E. coli* O157 Detection Kit
LightCycler® foodproof *Listeria Genus* Detection Kit
LightCycler® foodproof *Campylobacter* Detection Kit

Foodborne Pathogen Testing

Salmonella detection

A Validated Method



Extent of AOAC RI Approval

- Inclusivity-, exclusivity study
- Repeatability
- Ruggedness study
- Lot-to-lot study
- Stability study
- Independent testing

Foodborne Pathogen Testing

Salmonella detection

- **Suitable for various type of food matrices**
24 different food matrices were successfully tested
- **High sensitivity**
Detection of down to 5 copies of pure bacterial DNA
Detection of down to 10^3 cfu/ml of food enrichment culture
- **Specificity (exclusivity)**
60 bacteria phylogenetically close to *Salmonella* were tested → **all negative**

Specificity (inclusivity)

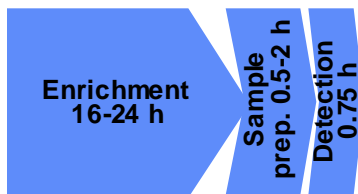
■ <i>Salmonella enterica</i> :	
Subspecies <i>enterica</i> (I)	552
Subspecies <i>salamae</i> (II)	42
Subspecies <i>arizonae</i> (IIIa)	27
Subspecies <i>diarizonae</i> (IIIb)	30
Subspecies <i>houtenae</i> (IV)	28
Subspecies <i>indica</i> (VI)	11
■ <i>Salmonella bongori</i>	17
Total: all detected	→ 707

Foodborne Pathogen Testing

Salmonella detection

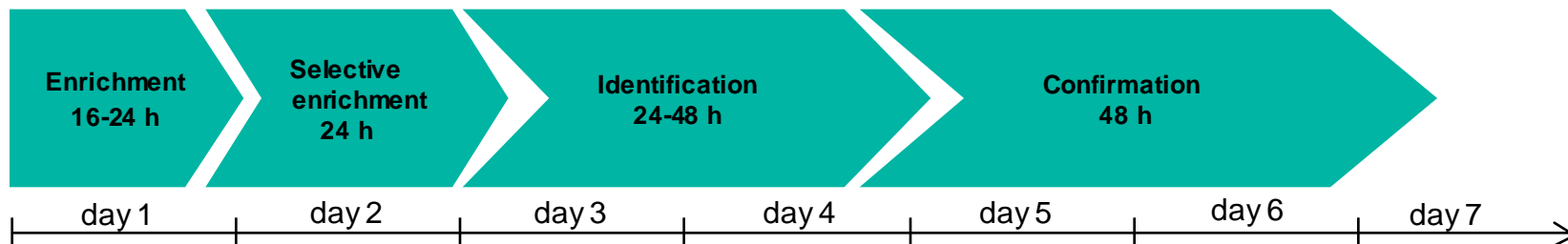
Time-to-result

≤ 2.5 days with PCR. No confirmation is necessary in case of positive results since a PCR control is amplified with each reaction.



≥ 3 days with traditional methods.

3-4 Days in case of negative results and > 6 days in case of positive results.



Foodborne Pathogen Testing

Listeria monocytogenes and *L. Genus*

A Validated Method

Detection of *Listeria monocytogenes*

Extent of AOAC RI Approval

- Inclusivity-, exclusivity study
- Repeatability
- Ruggedness study
- Lot-to-lot study
- Stability study
- Independent testing



Foodborne Pathogen Testing

Listeria monocytogenes and *L. Genus*

- **High specificity (inclusivity/exclusivity)**
The genus *Listeria* comprises of 6 species. Only the one human pathogen *L. monocytogenes* is tested positive
- **Suitable for various types of food matrices**
24 different food matrices were successfully tested
- **High Sensitivity**
Detection of down to 5 copies of pure bacterial DNA
Detection of down to 10³ cfu/ml of food enrichment culture

Inclusivity

- One human pathogen
Listeria monocytogenes **positive**
- One animal pathogen
Listeria ivanovii **negative**
(incl. subspecies *ivanovii* and *londoniensis*)
- None pathogen
Listeria grayi **all negative**
Listeria innocua
Listeria seeligeri
Listeria welshimeri

Exclusivity

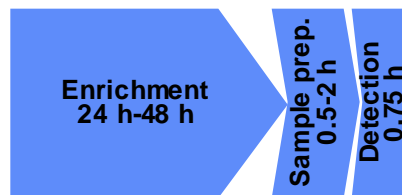
- 60 Bacteria phylogenetically close to *Listeria* genus were tested **all negative**

Foodborne Pathogen Testing

Listeria monocytogenes and *L. Genus*

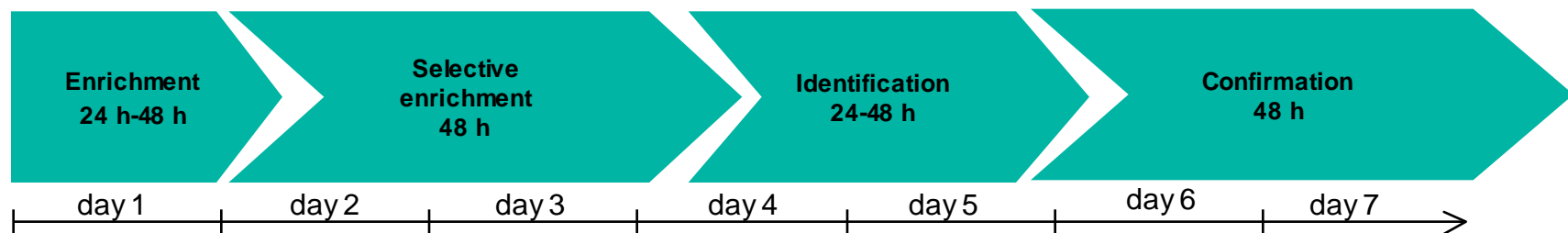
Time-to-result

≤ 2.5 days with PCR. No confirmation is necessary in case of positive results since a PCR control is amplified with each reaction.



≥ 5 days with traditional methods.

5 Days in case of negative results and > 7 days in case of positive results.





Foodborne Pathogen Testing

E. coli 0157 (incl. E. coli 0157)

A Validated Method



*coming
soon*

Extent of AOAC RI Approval

- Inclusivity-, exclusivity study
- Repeatability
- Ruggedness study
- Lot-to-lot study
- Stability study
- Independent testing

Foodborne Pathogen Testing

E. coli O157 (incl. *E. coli* O157)

- **High specificity (inclusivity/exclusivity)**
- **Suitable for various type of food matrices**
 - 24 different food matrices were successfully tested
- **High Sensitivity**
 - Detection of down to 5 copies of pure bacterial DNA
 - Detection of down to 10³ cfu/ml of food enrichment culture

Inclusivity

60 different *E. coli* O157 strains (incl. H7, H- und H16) were tested: **all positive**

38 *E. coli* O157:H7

19 *E. coli* O157:H-

2 *E. coli* O157:H16

1 *E. coli* O157 (H

Exclusivity

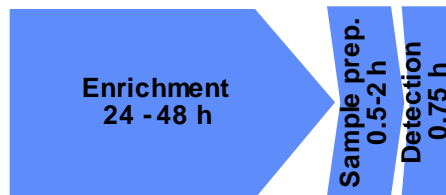
unknown)
> 60 different non-O157 *E. coli* strains were tested **all negative**

Foodborne Pathogen Testing

E. coli 0157 (incl. *E. coli* 0157)

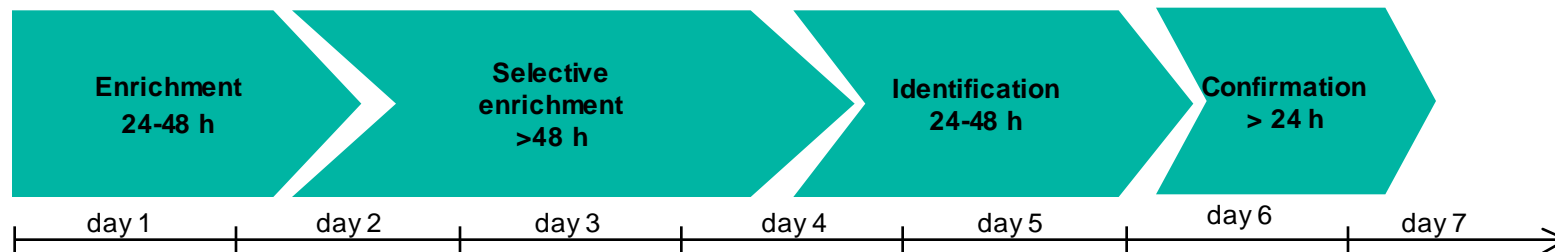
Time-to-result

≤ 2.5 days with PCR. No confirmation is necessary in case of positive results since a PCR control is amplified with each reaction.



≥ 5 days with traditional methods.

5 Days in case of negative results and > 6 days in case of positive results.



Foodborne Pathogen Testing

Campylobacter detection

A Validated Method



*coming
soon*

Extent of AOAC RI Approval

- Inclusivity-, exclusivity study
- Repeatability
- Ruggedness study
- Lot-to-lot study
- Stability study
- Independent testing

Foodborne Pathogen Testing

Campylobacter detection

- **High specificity: (inclusivity/exclusivity)**
Phylogenetically closely related bacteria strains (*Helicobacter* and *Arcobacter*) were tested → **all negative**
- **Suitable for various type of food matrices**
24 Different food matrices were successfully tested
- **High sensitivity**
Detection of down to 5 copies of pure bacterial DNA
Detection of down to 10^3 cfu/ml of food enrichment culture

Inclusivity

- Thermotolerant *Campylobacter* **all positive**
 114 x *C. jejuni*
 85 x *C. coli*
 4 x *C. lari*
 5 x *C. upsaliensis* **all positive**
- Other *Campylobacter*
 4 x *C. hyointestinalis*
 6 x *C. fetus*

Exclusivity

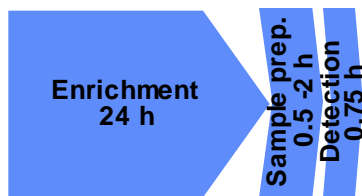
- 60 purified or non-purified DNA extracts of different bacteria strains were tested **all negative**

Foodborne Pathogen Testing

Campylobacter detection

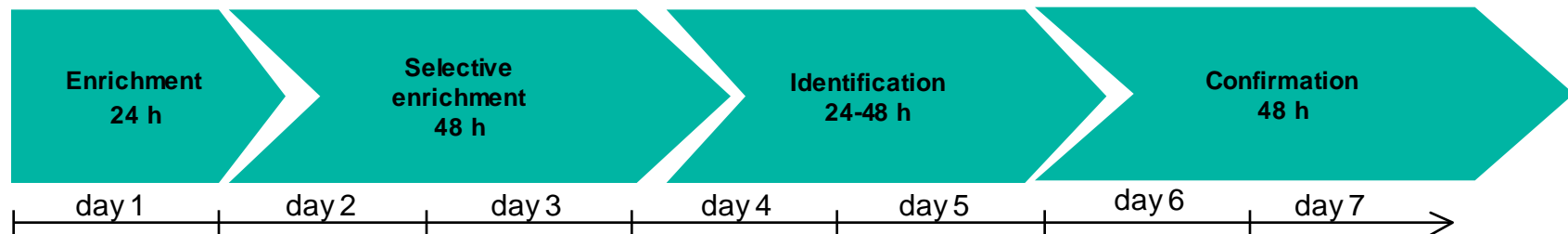
Time-to-result

≤ 1.5 days with PCR. No confirmation is necessary in case of positive results since a PCR control is amplified with each reaction.



≥ 5 days with traditional methods.

5 Days in case of negative results and > 7 days in case of positive results.





LightCycler® Systems

Open Channel

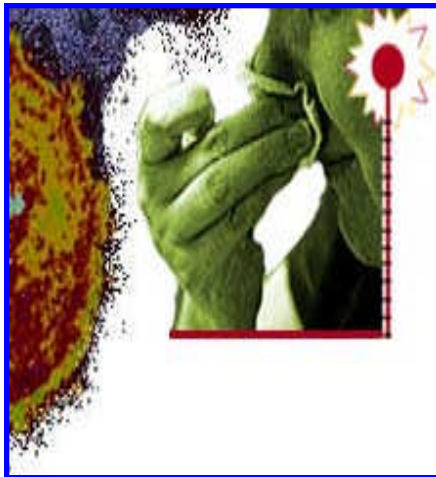
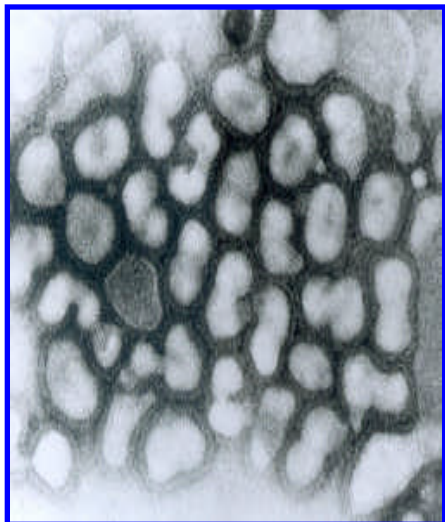


MOLBIOL

www.tib-molbiol.com



Diagnostics



AVIAN INFLUENZA VIRUS H5N1

Identification & Detection





AVIAN INFLUENZA VIRUS H5N1

LightCycler H5N1 Detection Set

- Set for the Quantitative Genomic Detection of the Avian Influenza Virus, Subtypes A H5 y A N1
- Technology Used: Real Time PCR using LightCycler v1.5 y v2.0 Systems
- Set Size: 96 Tests (including samples, standards and controls)
- Sample Material: **Aqueous Nucleic Acid Preparations.**
This Product is standardized only for Poultry

AVIAN INFLUENZA VIRUS H5N1



Diagnosics

LightCycler H5N1 Detection Set : Components

Biological sample extraction kit.

High Pure RNA Isolation Kit No. 11 828 665 001

Retrotranscription kit.

Transcriptor First Strand cDNA Synthesis Kit -No. 04 379 012 001

Thermal cycler.

LightCycler 2.0 System No. 03 531 414 201

LightCycler 1.5 System No. 04484495 001



Amplification enzyme and related reagents

LightCycler® FastStart^{PLUS} DNA Master Hybridization Probes -No. 03 515 575 001

Amplification specific reagents and PCR protocols

LightMix Influenza virus A H5

LightMix Influenza virus A N1

-No. 40-0219-16

-No. 40-0230-16



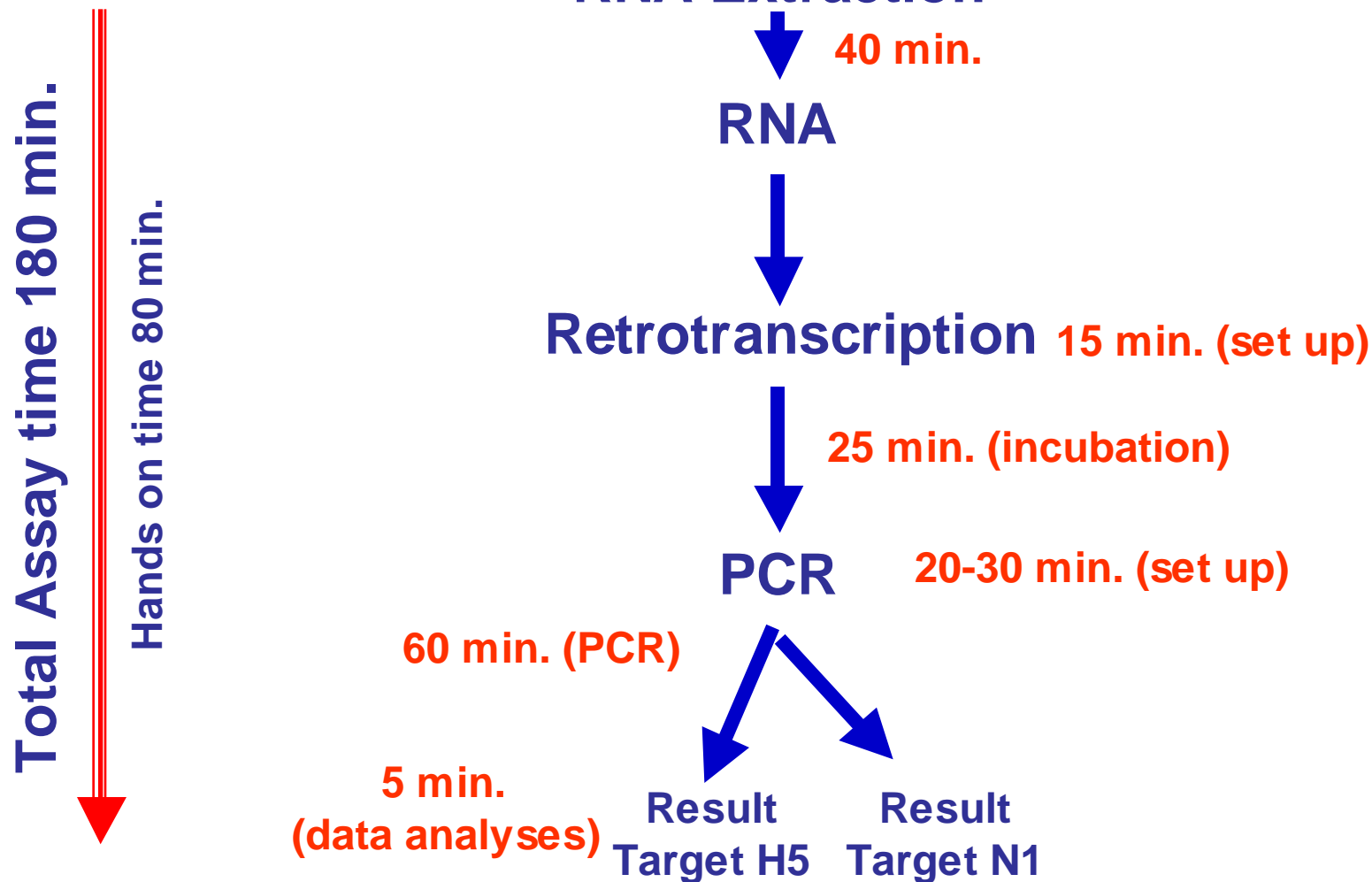
Diagnosics



AVIAN INFLUENZA VIRUS H5N1



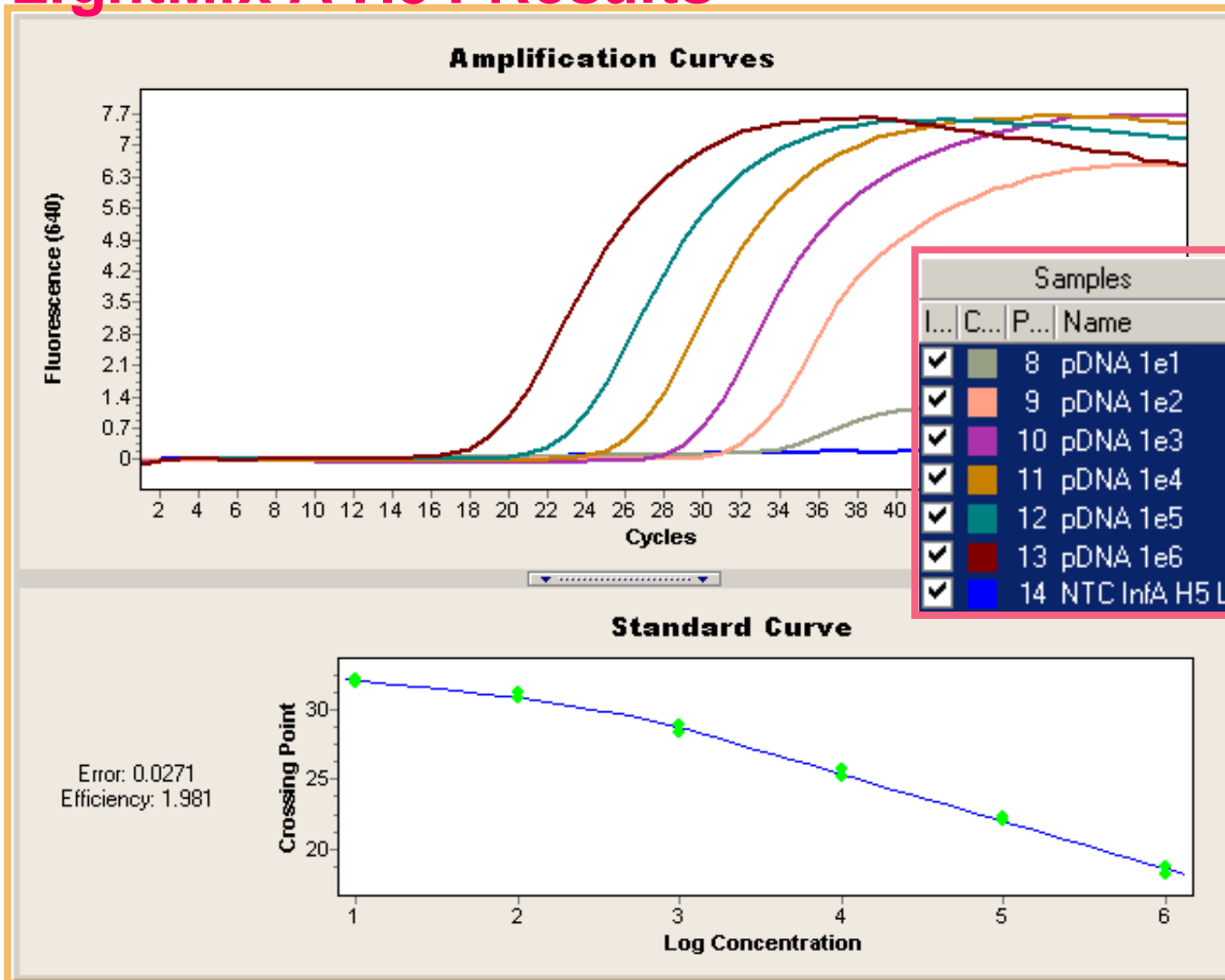
LightCycler H5N1 Detection Set : Work Flow



AVIAN INFLUENZA VIRUS H5N1



LightMix A H5 : Results

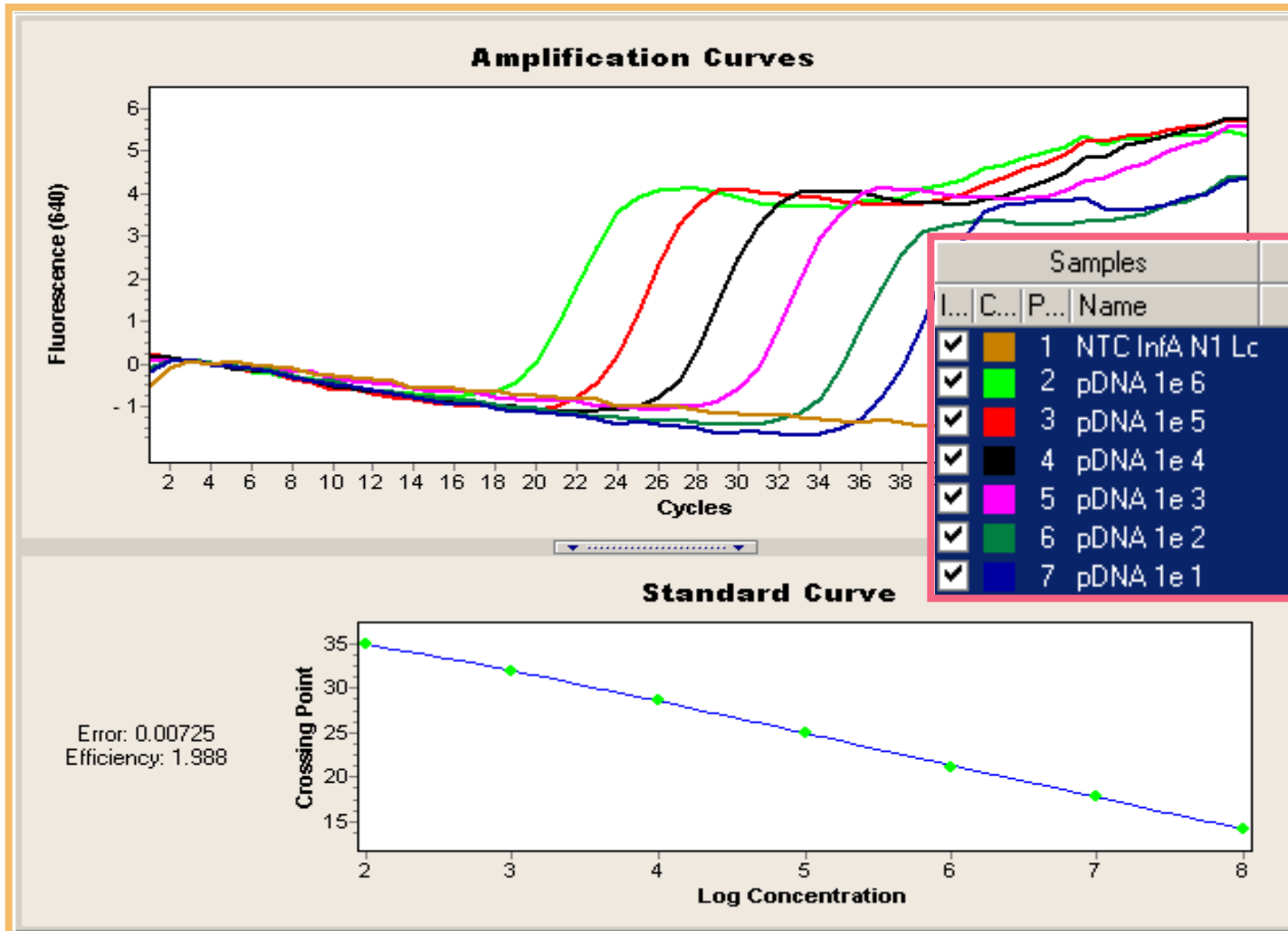


Samples			Results			
I...	C...	P...	Name	CP	Conc...	Standard
<input checked="" type="checkbox"/>	8		pDNA 1e1	32.18	8.64E0	1.00E1
<input checked="" type="checkbox"/>	9		pDNA 1e2	31.21	5.18E1	1.00E2
<input checked="" type="checkbox"/>	10		pDNA 1e3	28.37	1.29E3	1.00E3
<input checked="" type="checkbox"/>	11		pDNA 1e4	25.25	1.09E4	1.00E4
<input checked="" type="checkbox"/>	12		pDNA 1e5	22.04	9.79E4	1.00E5
<input checked="" type="checkbox"/>	13		pDNA 1e6	18.22	1.33E6	1.00E6
<input checked="" type="checkbox"/>	14		NTC InfA H5 Lc			

AVIAN INFLUENZA VIRUS H5N1



LightMix A N1 : Results



Samples				Results		
I...	C...	P...	Name	CP	Conc...	Standard
<input checked="" type="checkbox"/>			1 NTC InfA N1 Lc			
<input checked="" type="checkbox"/>			2 pDNA 1e 6	17.75	9.60E 5	1.00E 6
<input checked="" type="checkbox"/>			3 pDNA 1e 5	21.15	1.10E 5	1.00E 5
<input checked="" type="checkbox"/>			4 pDNA 1e 4	24.98	9.68E 3	1.00E 4
<input checked="" type="checkbox"/>			5 pDNA 1e 3	28.58	9.86E 2	1.00E 3
<input checked="" type="checkbox"/>			6 pDNA 1e 2	31.99	9.74E 1	1.00E 2
<input checked="" type="checkbox"/>			7 pDNA 1e 1	34.99	1.01E 1	1.00E 1

Laboratory Set Up

Two areas : Pre-PCR and Post PCR

- Pre-PCR: Reagent Preparation



PCR Enclosure
with UV



Micropipettes 20,
200 y 1000 uL



Refrigerator

Laboratory Set Up

- Pre-PCR: Sample Extraction



Laminar Flow Cabinet



Centrifuge



Micropipettes 20,
200 y 1000 μ L



Vortex



Heating Block 1.5 – 2.0 mL
and 95 – 100 $^{\circ}$ C

Laboratory Set Up

- Post-PCR: Amplification & Detection



LightCycler Instrument



PC + Color Printer

Summary

Fully Automated Solution: *LightCycler® system*



LightCycler® System

- Two versions available:
 - version 1.5: three channels
 - version 2.0: six channels (Multiplex Analysis)
- Rapid PCR performance
 - High-speed thermal cycling using air as temperature transfer medium
 - 1-32 Samples with highest reproducibility
 - Complete PCR (30-40 cycles) within 20-30 minutes
- Real-time and online monitoring
- Flexibility
 - Assay format
- LightCycler® Software
 - Automatic execution and analysis
 - Simplified data and user management

Summary

Food Testing Integrated Solutions: *A complete workflow*

*“Speed up food product analysis using
Roche Diagnostics Integrated PCR Solutions”*

■ Fast

- Gain several days in the implementation of corrective actions on the production line.
- Generate significant cost savings by speeding product release and achieving a quicker turnover.

■ Easy-to-use

- Test set-up and procedures are simple and suitable for daily routine lab testing.
- Standardized test procedure and automated results interpretation.

■ Reliable

- Highly accurate results using high specific and sensitive method.
- No risk of false positive and negative results.
- No confirmation required (in case of positive results).
- AOAC approved for pathogen testing.

■ Wide Portfolio: Pathogens, Open Channel: **Avian Influenza**



Food Testing Integrated Solutions

Further information

■ Internet

http://www.roche-applied-science.com/molecular-food-safety-testing/homepage_frameset.htm

LightCycler® special interest site

www.lightcycler-online.com

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Fax (+507) 227 7977



Thank You for Your Attention

www.roche-applied-science.com/molecular-food-safety-testing



Diagnostics

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