

The background of the slide is a microscopic image showing a dense array of pinkish, hair-like cilia. Interspersed among these cilia are several bright yellow, rod-shaped structures, which are likely bacteria. The overall appearance is that of a biological surface, possibly the respiratory tract, under high magnification.

Laboratory Diagnosis of Pertussis (whooping cough)

ANUSAK KERDSIN

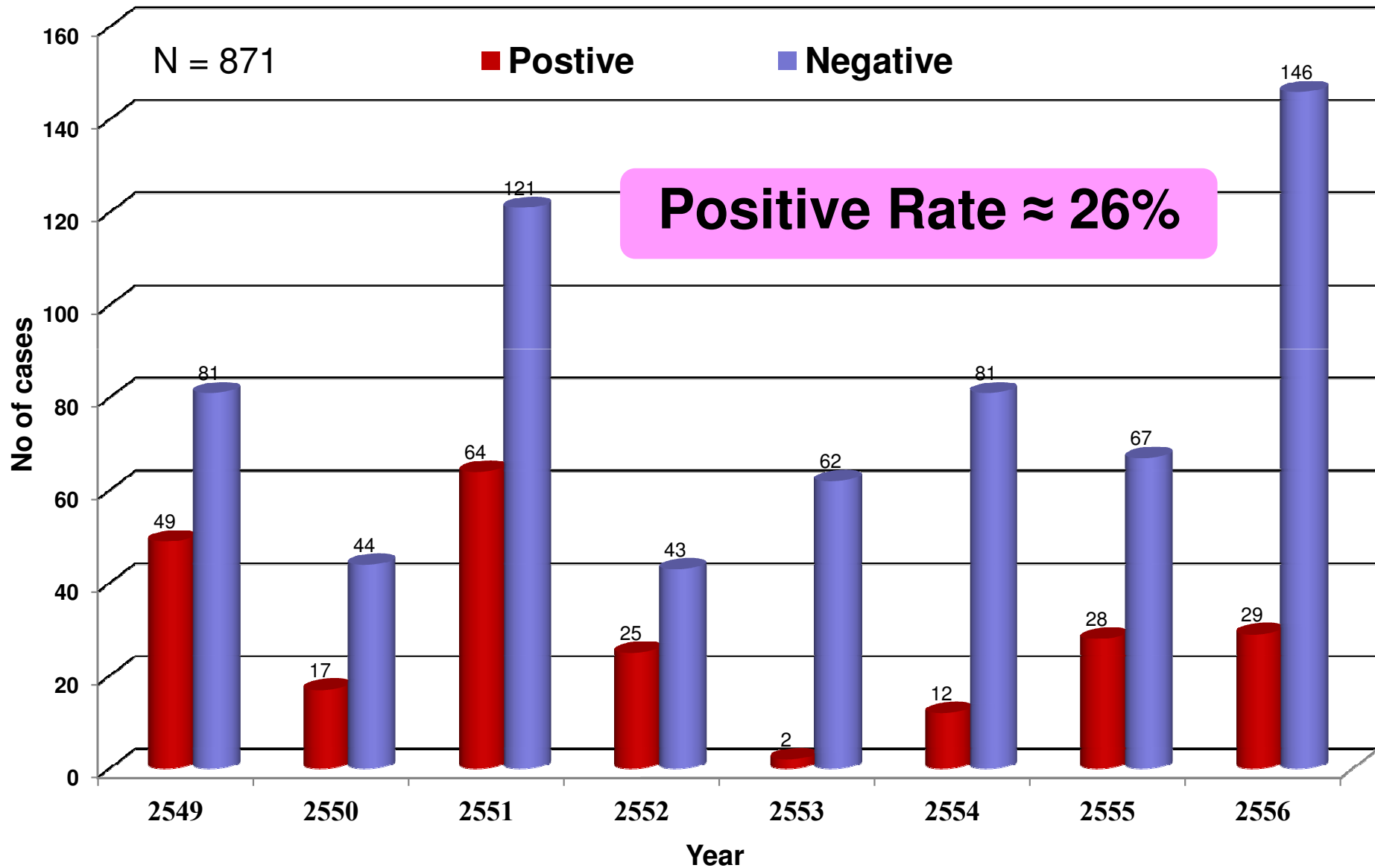
**National Institute of Health
Department of Medical Sciences
Ministry of Public Health**

Bordetella

Species	Host	Transmission	Diseases
<i>B. pertussis</i>	Humans	Droplets	Pertussis
<i>B. parapertussis</i>	Humans	Droplets	Pertussis-like disease
	Sheep	Unknown	Respiratory disease
<i>B. bronchiseptica</i>	Humans	Droplets (?)	Respiratory disease Systemic infection *
	Animals	Droplets (?)	Respiratory disease
<i>B. hinzii</i>	Humans	Unknown	Cholangitis, arthritis
	Poultry	Droplets	Respiratory disease
<i>B. trematum</i>	Humans	Unknown	Wound infection, otitis
<i>B. holmesii</i>	Humans	Unknown	Pertussis-like illness, Systemic infection*
<i>B. petrii</i>	Humans	Unknown	Osteomyelitis, mastoiditis
	Environment		
<i>B. avium</i>	Humans	Unknown	Respiratory disease
	Poultry	Droplets	Respiratory disease
<i>"B. ansorpii"</i>	Humans	Unknown	Epidermal cyst, Systemic infection*

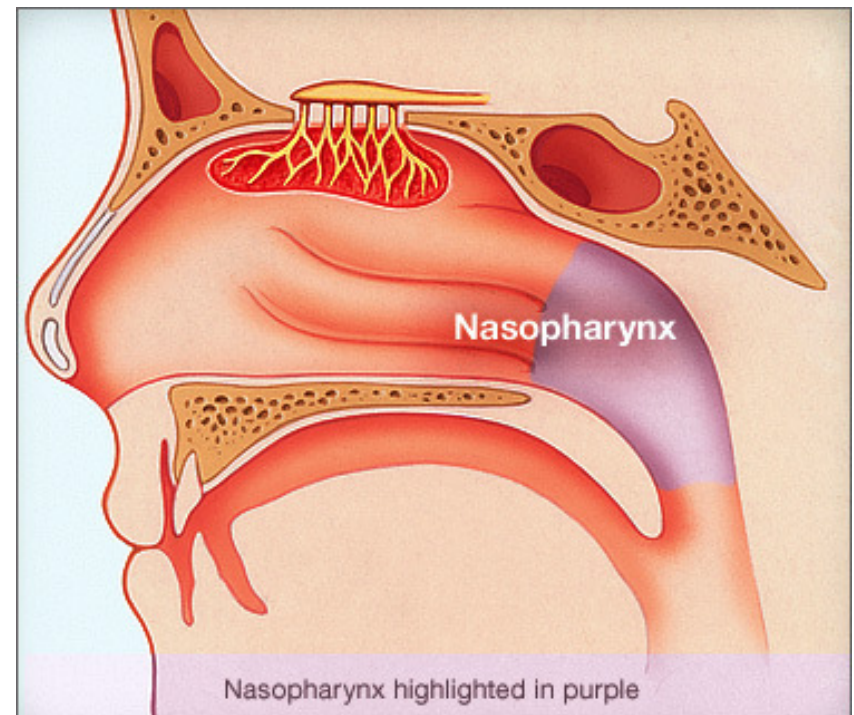
* Immunocompromised hosts

Laboratory Detection of Pertussis by Thai-NIH



Specimen collection for pertussis diagnosis

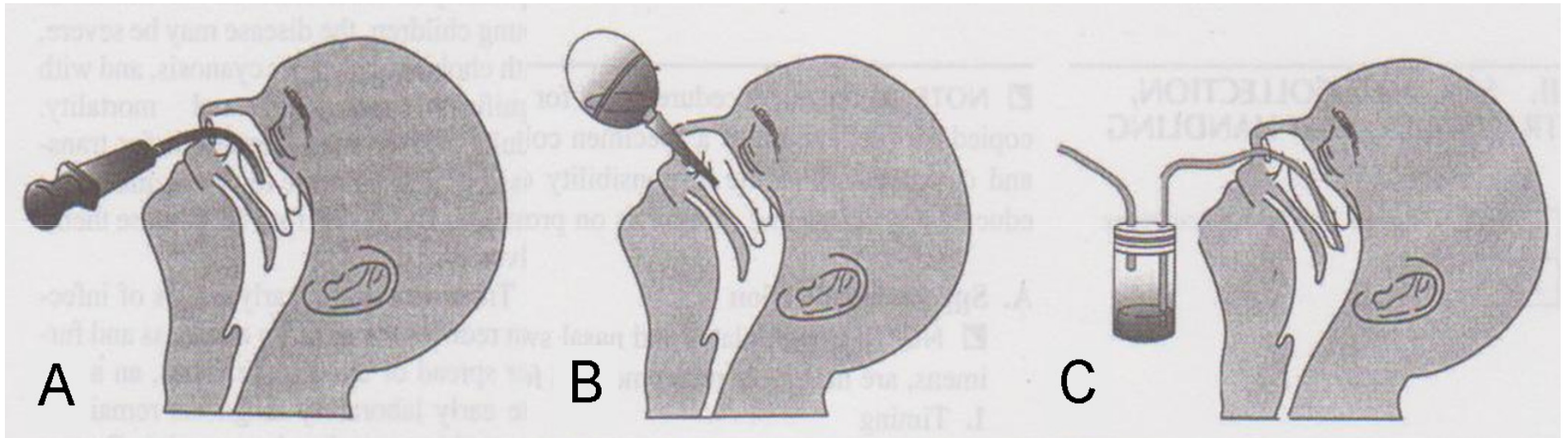
- For *B. pertussis* detection
 - Nasopharyngeal aspirate/wash
 - Nasopharyngeal swab
- For serology testing (Ab titer)
 - Serum



Specimen collection

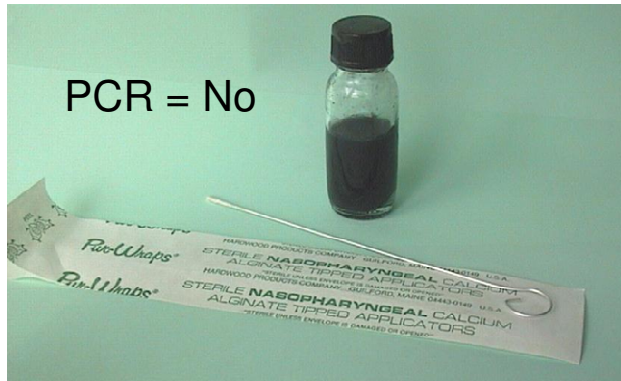
Specimens	Culture	DFA	PCR
NP aspirate	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Cotton swab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Calcium alginate swab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Polyester: Dacron swab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Rayon swab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Flocked swab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Specimen collection : Nasopharyngeal aspirates

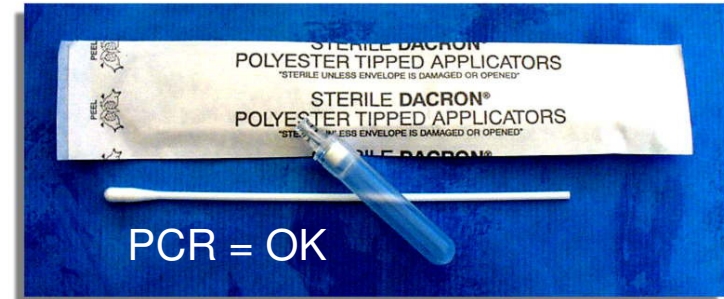


Sterile technique !!!

Specimen collection : Nasopharyngeal swab

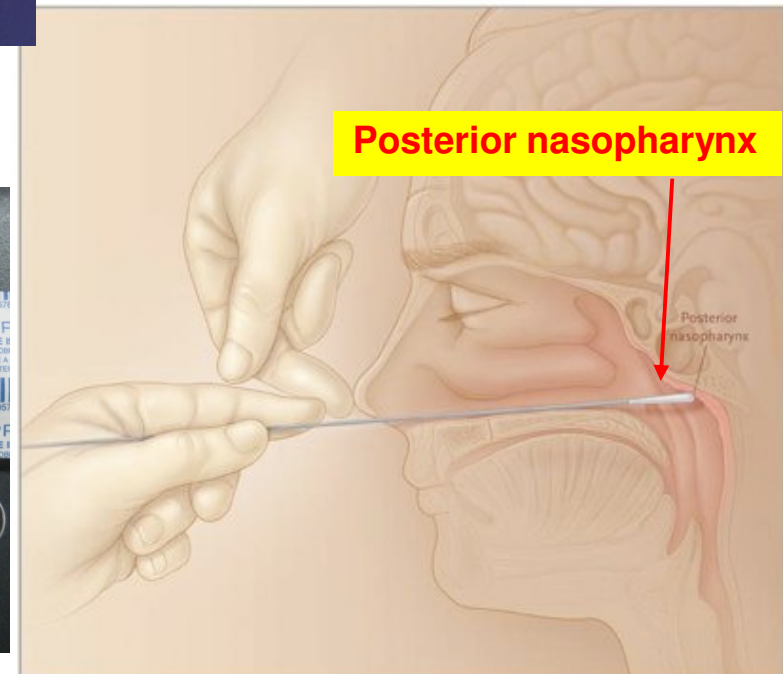
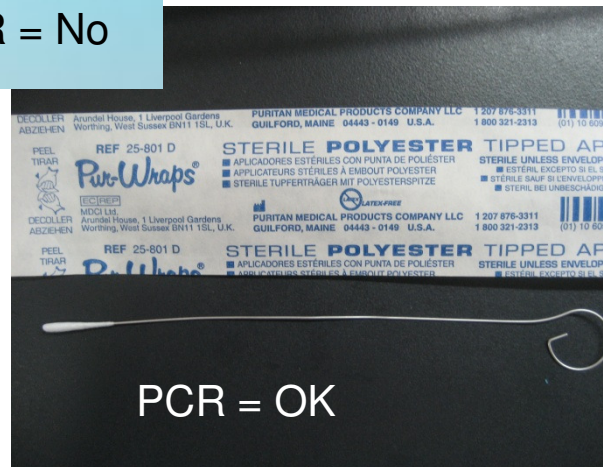


Nylon-flocked swab



The swab tips may be **polyester** (such as Dacron®), **rayon**, or **nylon-flocked**.

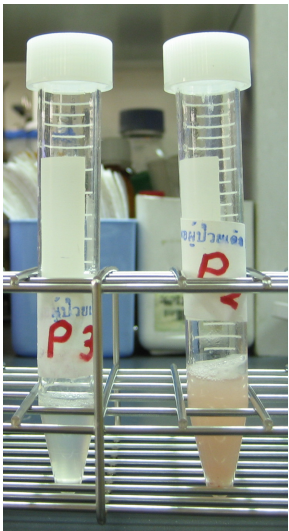
Cotton-tipped or calcium alginate swabs are not acceptable as residues present in these materials inhibit PCR assays.



Specimen collection and transportation to Thai-NIH

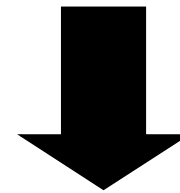


+ แช่เย็น หรือ ไม่แช่ ก็ได้



+ แช่เย็น หรือ แช่แข็ง เท่านั้น

นำส่งภายใน 24-48 ชั่วโมง



โครงการ VPIS ฝ่ายแบคทีเรียทั่วไป
สถาบันวิจัยวิทยาศาสตร์สาธารณสุข
กรมวิทยาศาสตร์การแพทย์
88/7 ถนนติวานนท์ อำเภอเมือง
จังหวัดนนทบุรี 11000
โทร.0-2589-9850, 0-2951-0000-11
ต่อ 99404, 99409, 99415
หรือโทร 02-951-1486

Laboratory diagnosis of pertussis

- **Serology**: ELISA (anti-PT Ab)
- **Culture**: Special media = Regan-Lowe medium, Bordet-Gengou medium, Stainer-Scholte medium
- **DFA**
- **Molecular**: PCR, real-time PCR, DNA hybridization, DNA sequencing

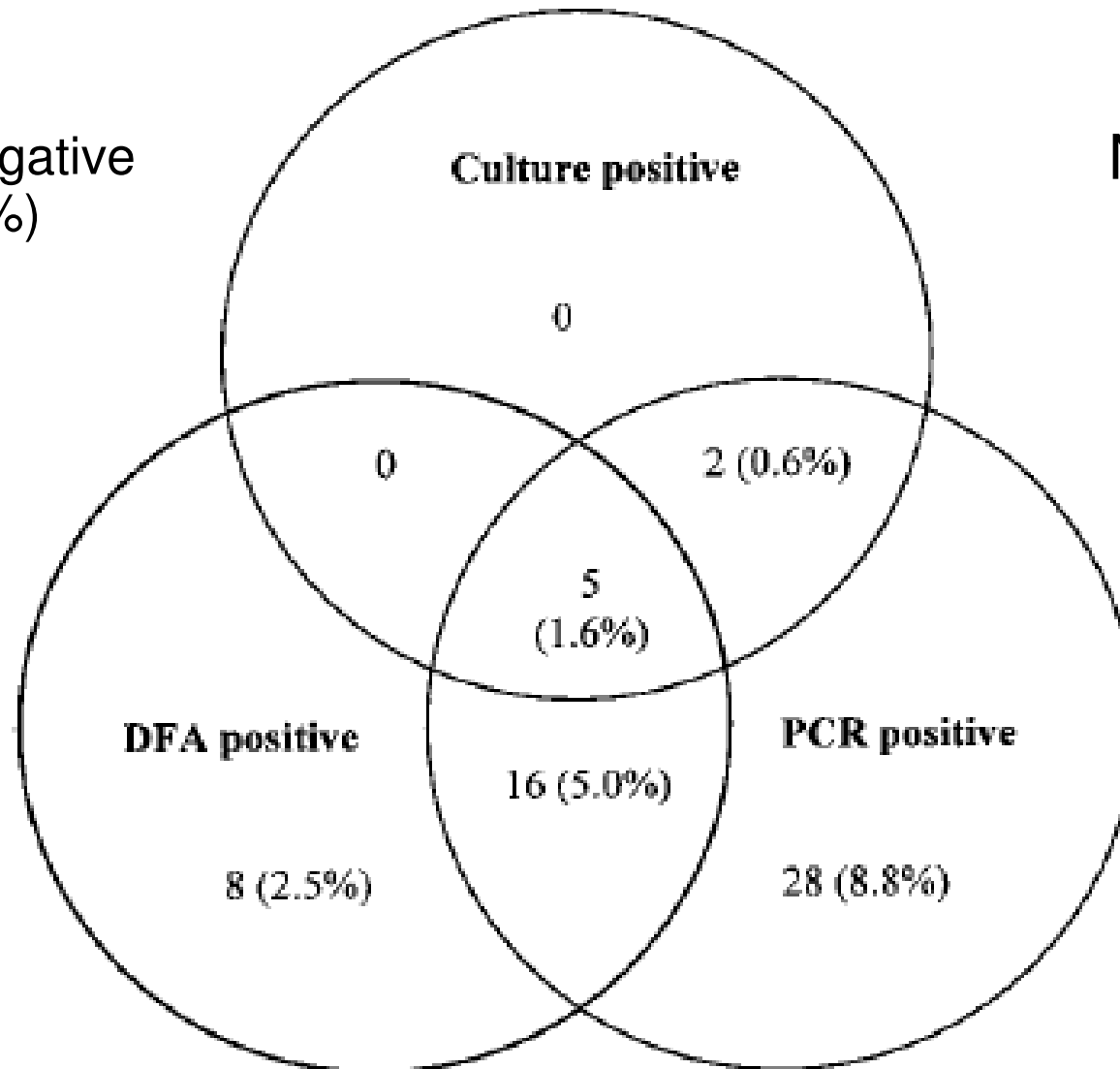
Laboratory testing for pertussis diagnosis

Test	Sensitivity (%)	Specificity (%)
Culture	12-60	100
DFA	11-68	99-100
PCR	70-99	86-100
Serology: PT Ab	50-99 by >3 wks after cough onset	>90
Serology: FHA Ab	50-99 by >3wks after cough onset	≈50

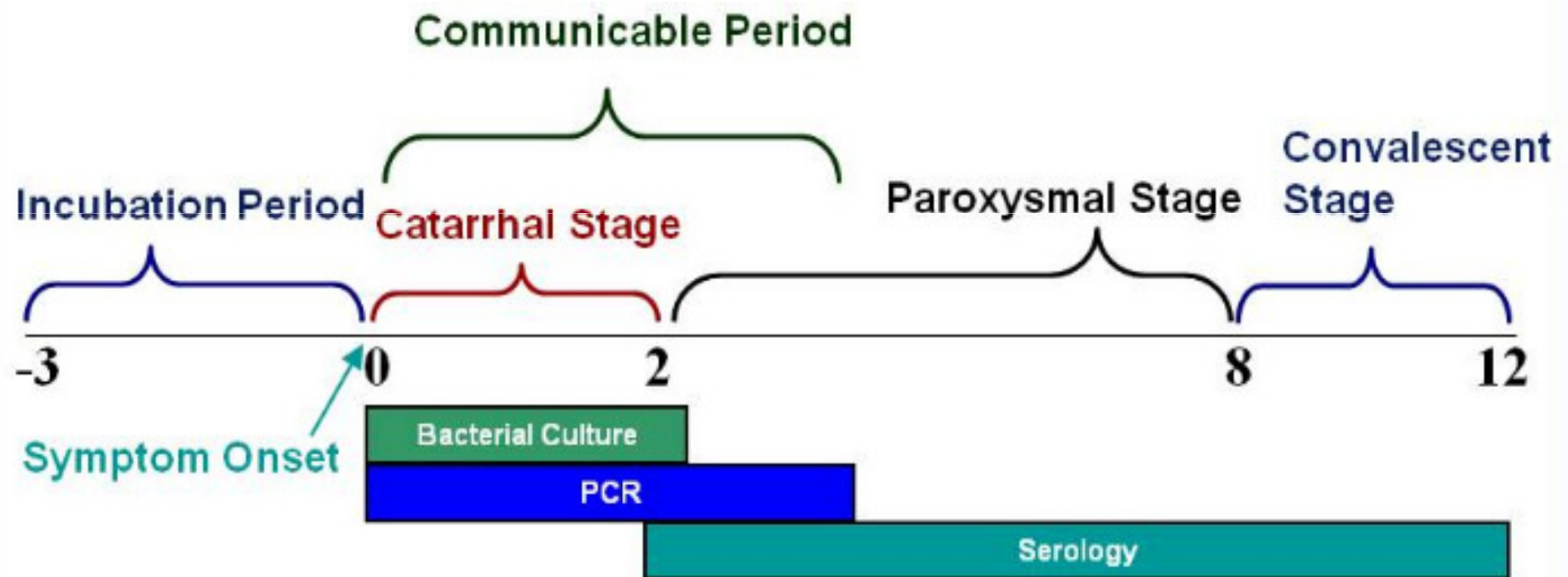
Agreement among PCR, Culture, DFA

All tests negative
260 (81.5%)

N = 319



Optimal Timing for Diagnostic Testing



Laboratory diagnosis of pertussis

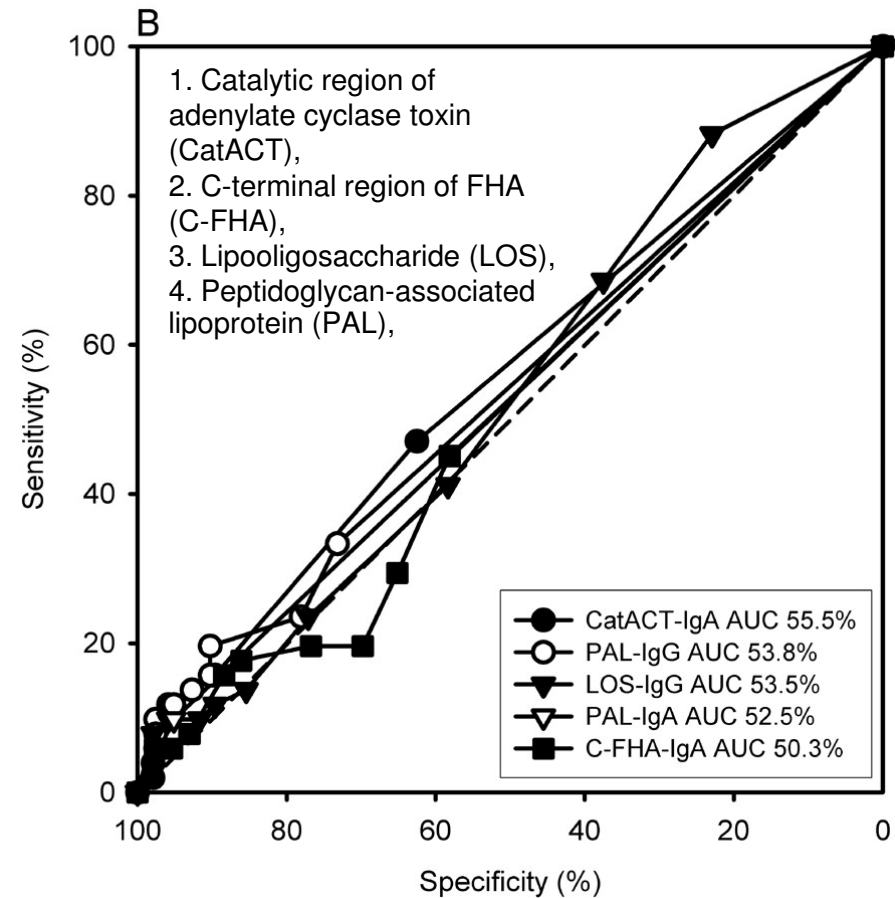
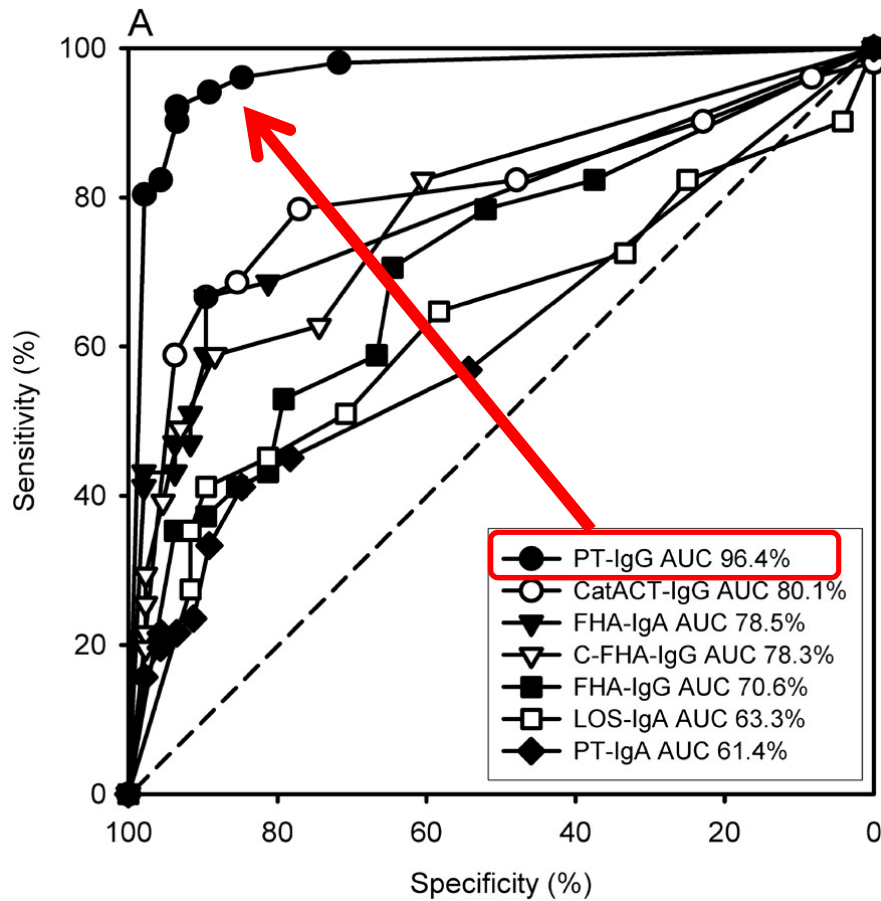
- **Serology**
- **Culture**
- **DFA**
- **Molecular**

Serology

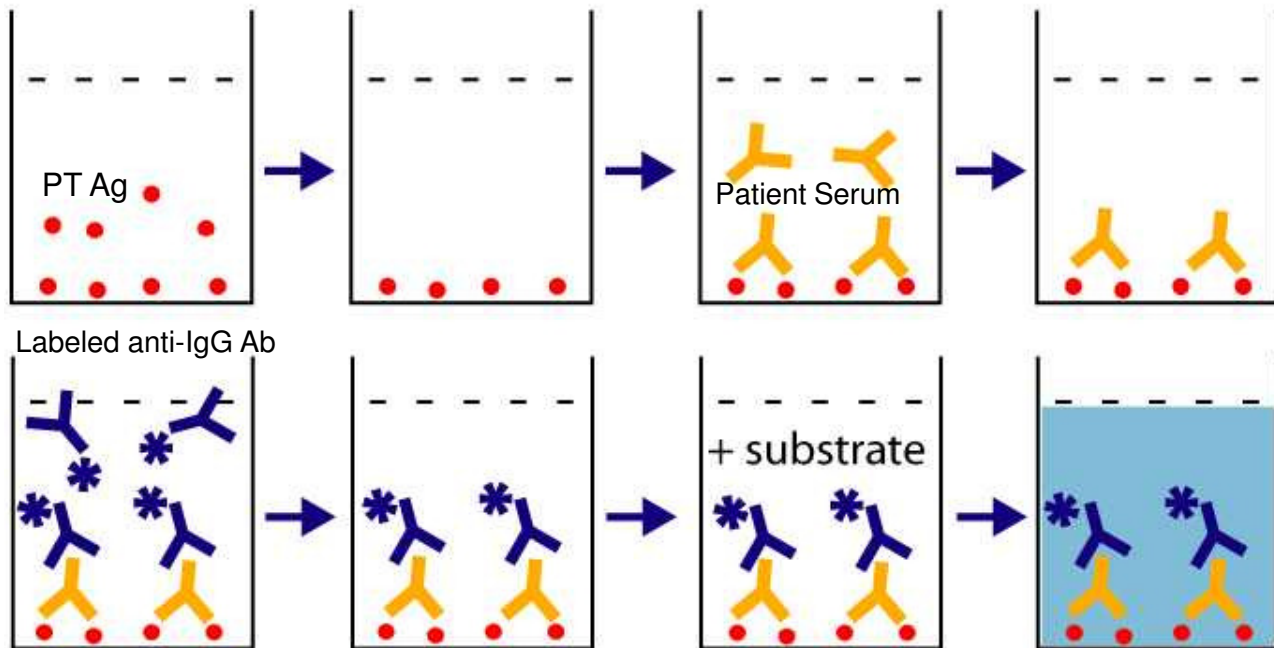
- Originally designed for vaccine evaluation.
- A useful method for diagnosing pertussis especially in adults and in the later stages of the disease.
- Useful for confirming diagnosis.
- 2 targets: pertussis toxin (PT) & filamentous hemagglutinin (FHA)

Serology

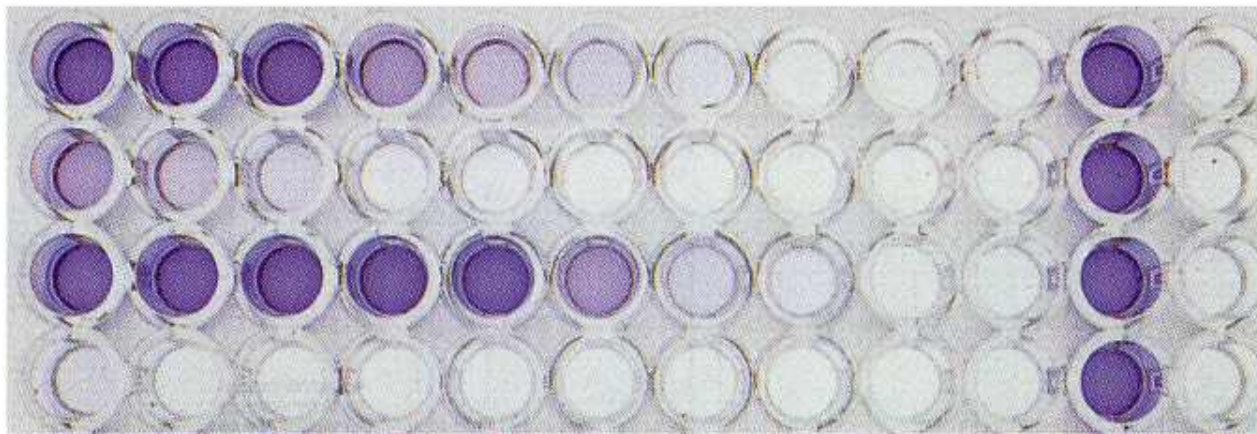
IgG against pertussis toxin (PT) is the most specific and sensitive target



Serology



The concentration of the PT-IgG Ab is directly proportional to the intensity of the color.



Laboratory diagnosis of pertussis

- **Serology**
- **Culture**
- **DFA**
- **Molecular**

Culture

- Gold standard
- 100% specific, but low sensitivity
 - Most sensitive within first two weeks after cough onset.
- Highest yield
 - Young patients.
 - Unvaccinated patients
 - Patients early in cough illness prior to antimicrobials.

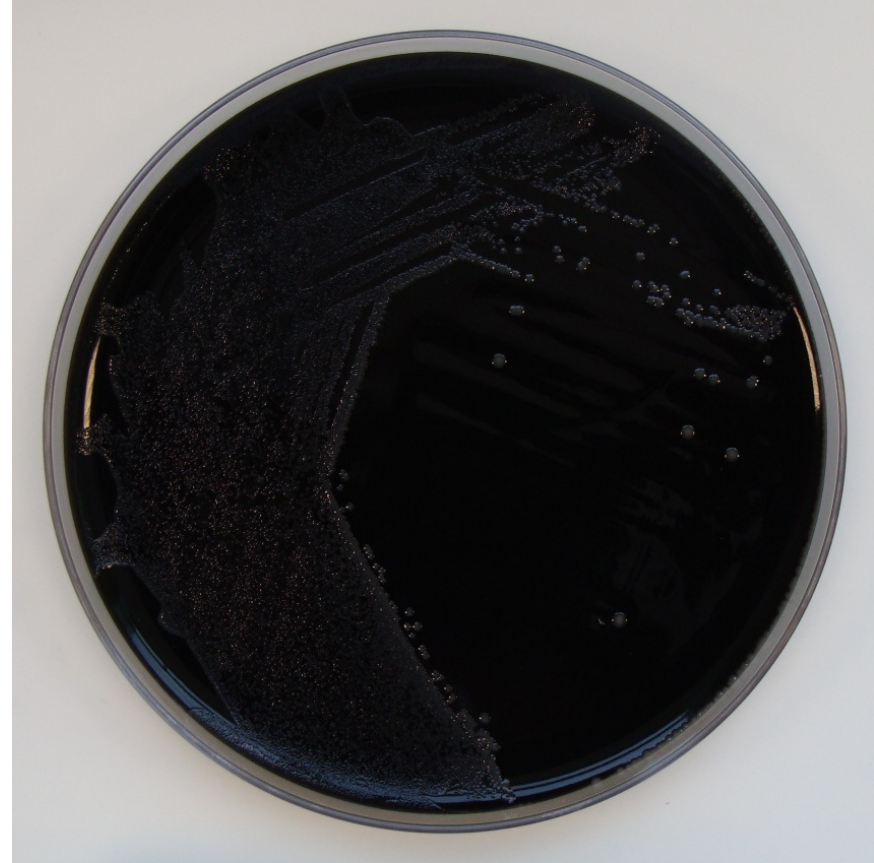
Culture

- Media + Antibiotic (Cephalexin)
 - **Regan-Lowe charcoal agar**: higher sensitivity, rapid growth, higher number of colonies
 - Bordet-Gengou agar
 - Jones-Kendrick charcoal agar
 - Stainer-Scholte synthetic medium
- Incubation
 - 35°C + high humidity (moisture chamber)
 - CO₂ is not needed.
 - 3-12 days

***B. pertussis* colony**

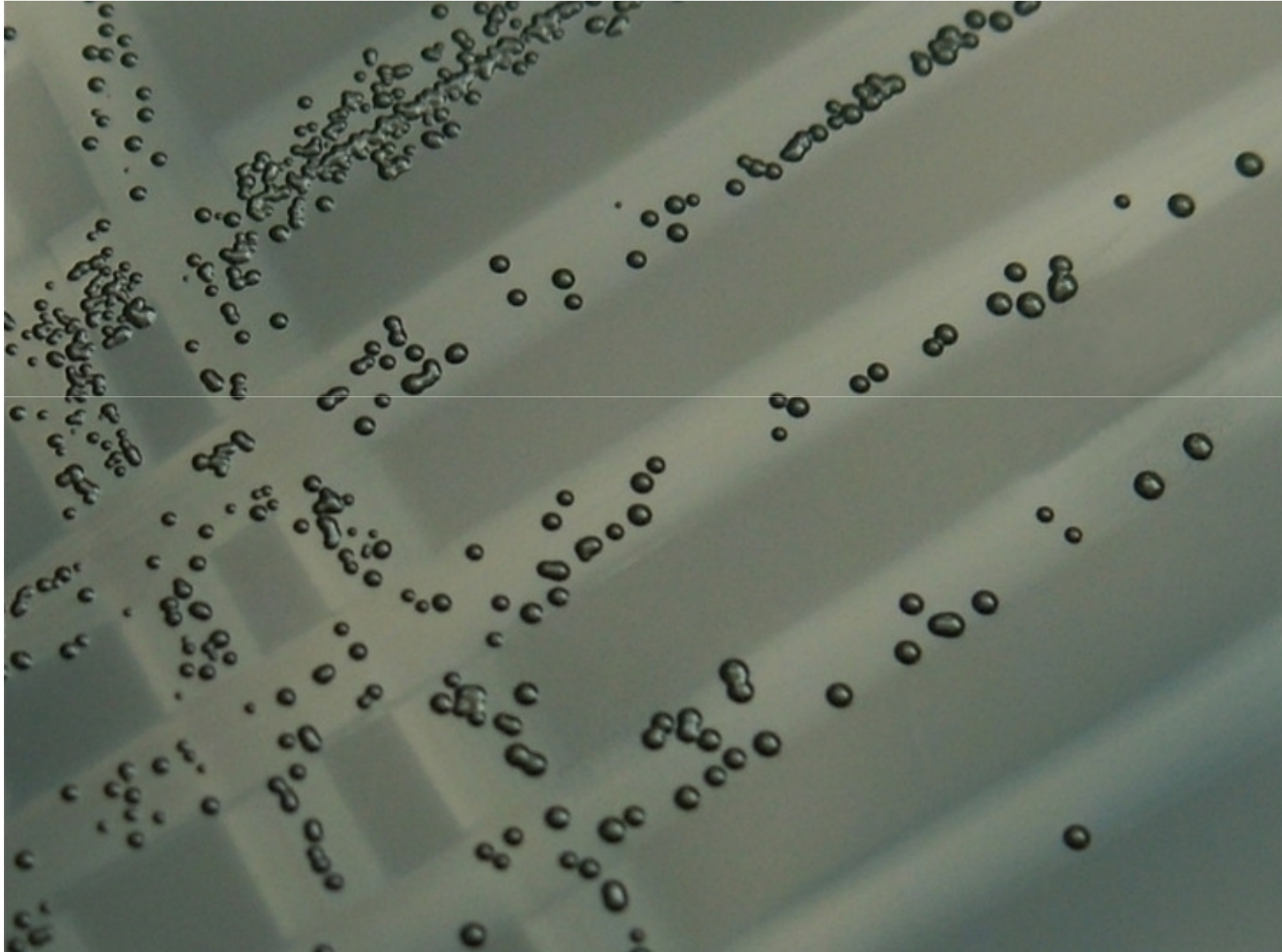


Bordet-Gengou agar



**Regan-Lowe agar
(Charcoal blood agar)**

B. pertussis colony on Regan-Lowe medium



Biochemical characteristics

TABLE 3 Useful characteristics for differentiating *Bordetella* species

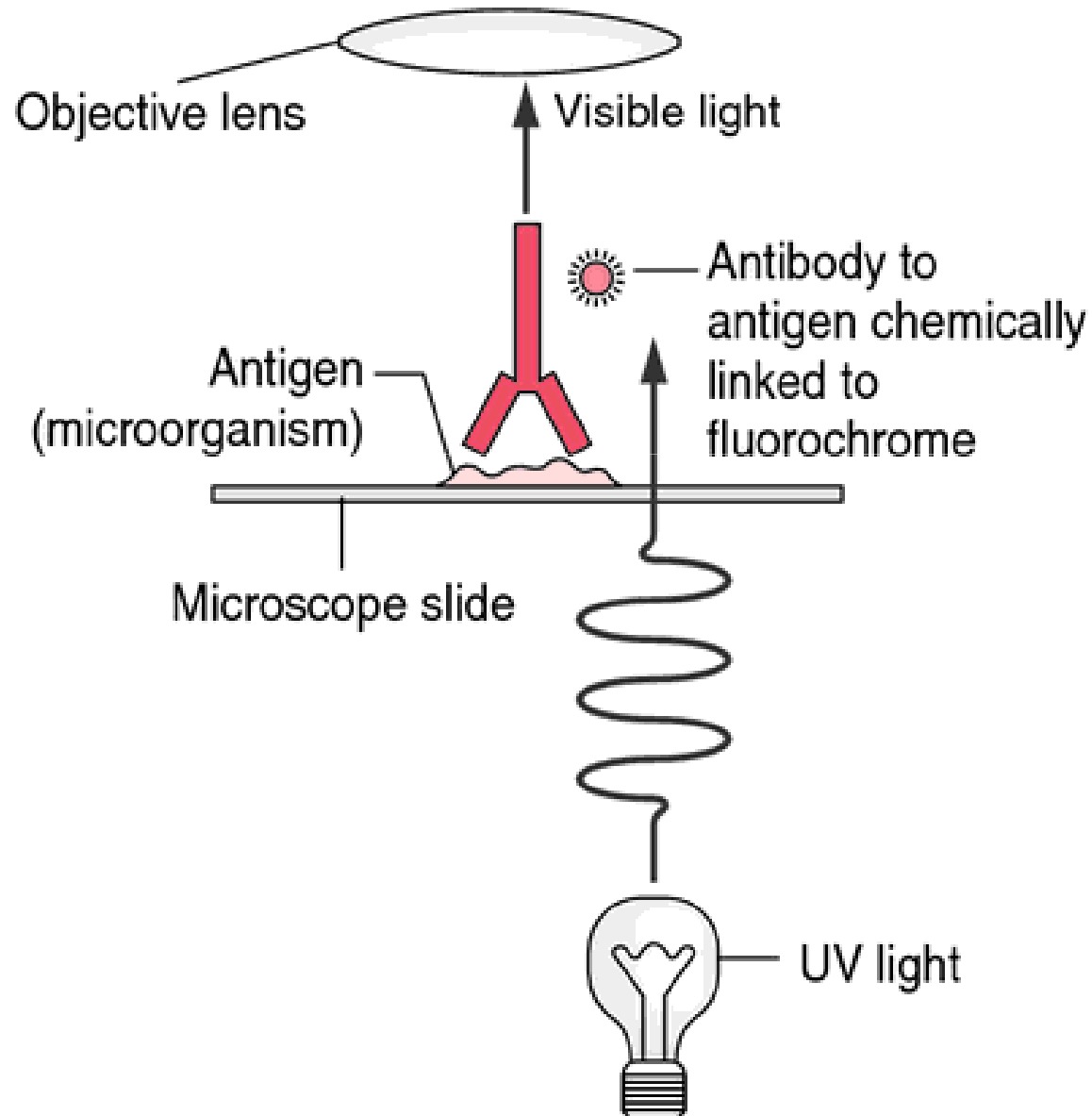
Characteristic ^a	<i>B. pertussis</i>	<i>B. parapertussis</i>	<i>B. bronchiseptica</i>	<i>B. avium</i>	" <i>B. ansorpii</i> "	<i>B. hinzii</i>	<i>B. holmesii</i>	<i>B. petrii</i>	<i>B. trematum</i>
Growth on:									
RL medium	3–4 days	2–3 days	1–2 days	ND	ND	ND	ND	ND	ND
Columbia agar	–	V	+	+	+	+	+	+	+
MacConkey agar	–	–	+	+	+	+	V	+	+
Catalase	+	+	+	+	+	+	V	+	+
Oxidase	+	–	+	+	+	+	–	+	–
Motility	–	–	+	–	V	+	–	–	+
Pigment	–	Brown	–	–	–	–	–	Yellow	Yellow
Reduction of:									
Nitrate	–	–	+	–	–	–	–	+	V
Urea	–	+	+	–	–	–	–	–	–
Citrate	–	–	V	V	V	+	–	+	+

^aSymbols and abbreviations: +, ≥90% of the strains are positive; –, ≤10% of the strains are positive; V, 10 to 89% of the strains are positive; ND, not determined.

Laboratory diagnosis of pertussis

- **Serology**
- **Culture**
- **DFA**
- **Molecular**

Direct Immunofluorescence Assay (DFA)



- Has been in use since 1960.
- Presumptive diagnosis
- Low sensitivity (11-68%)
- 2 commercial reagents:
pAb & mAb



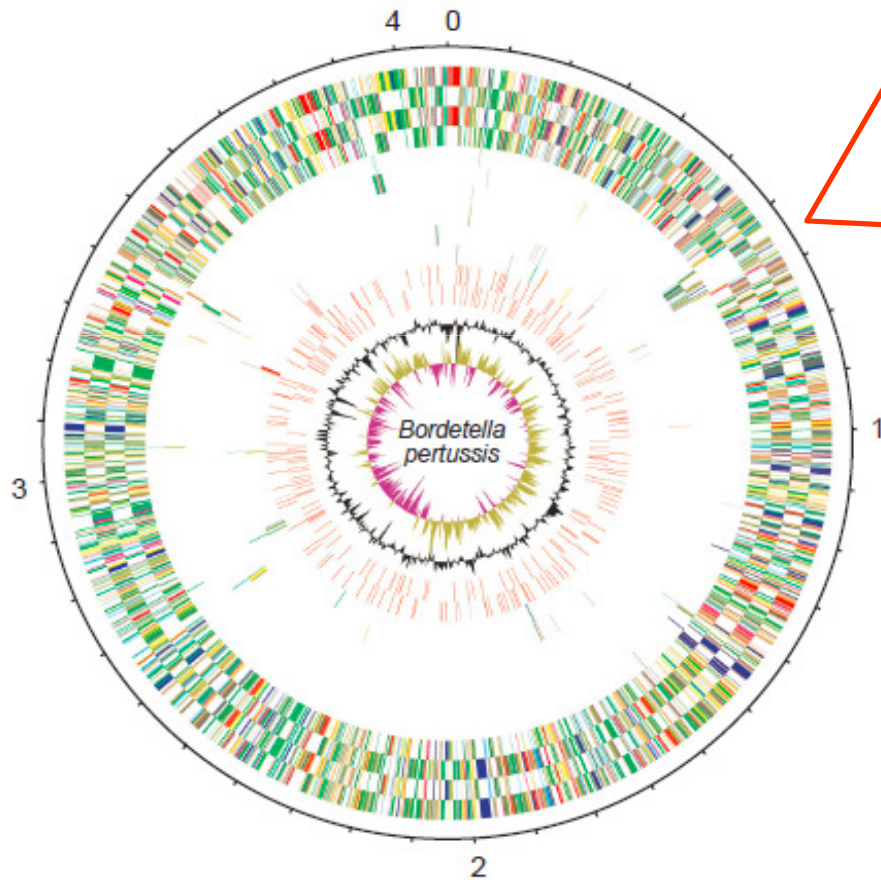
Laboratory diagnosis of pertussis

- **Serology**
- **Culture**
- **DFA**
- **Molecular**

Molecular methods for pertussis diagnosis

- Nucleic acid amplification
 - PCR
 - Real-Time PCR
 - LAMP (Loop-Mediated Isothermal Amplification)
- DNA sequencing
- DNA-DNA hybridization

***B. pertussis* –
chromosomal target region
for PCR**



1. Promoter region of pertussis toxin
2. Repeated insertion sequence : IS481 for *B. pertussis*, IS1001 for *B. parapertussis*
3. Upstream region of the porin gene
4. Adenylate cyclase gene
5. Filamentous hemagglutinin gene
6. Flagellin gene
7. Pertactin gene
8. recA gene
9. Unknown function: BP283, BP485, BP3385

Bordetella insertion sequences

Insertion sequence	Presence / no. of copies per genome			
	<i>B. pertussis</i>	<i>B. parapertussis</i>	<i>B. holmesii</i>	<i>B. bronchiseptica</i>
<i>IS481</i>	+ / >50	- / NA	+ / 8-10	(+) / ND
<i>IS1001</i>	- / NA	+ / 20	- / NA	(+) / 1-7
<i>hIS1001</i>	- / NA	- / NA	+ / 3-5	- / NA
<i>IS1002</i>	+ / 4-8	+ / 9	- / NA	- / 1

+ = present in all isolates
 (+) = present in some isolates
 - = absent from all isolates
 NA = not applicable
 ND = not determined

Real-time PCR in Thai-NIH

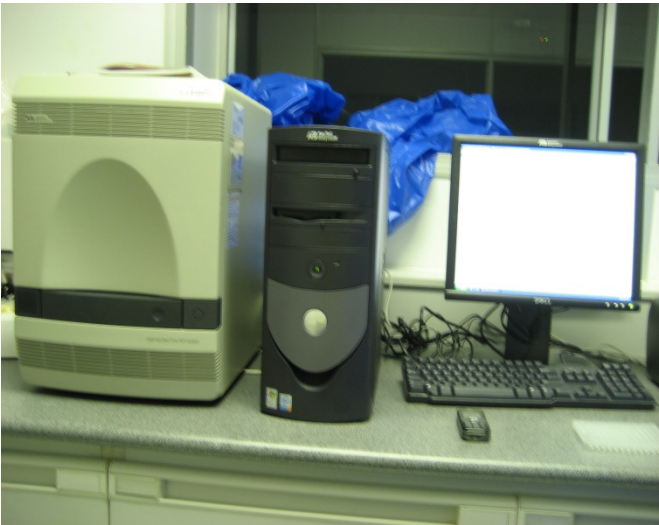
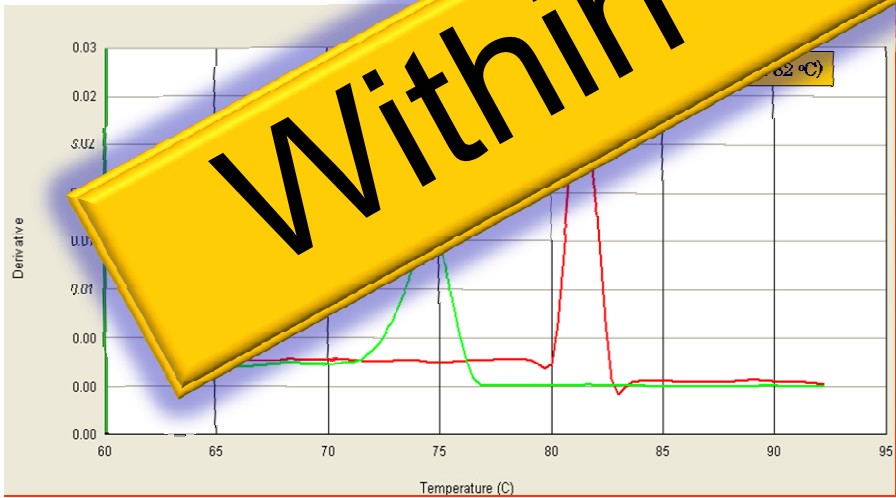
รายการทดสอบ: การตรวจหาสารพันธุกรรมเชื้อ
Bordetella pertussis ด้วยเทคนิค PCR

- ☑ Certification: ISO15189
- ☑ Technique: Real-Time PCR
- ☑ Laboratory processing: ≤ 3 days

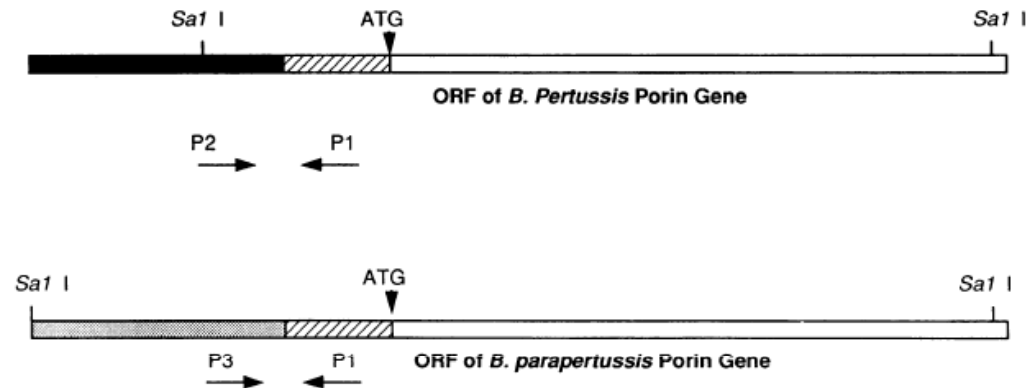
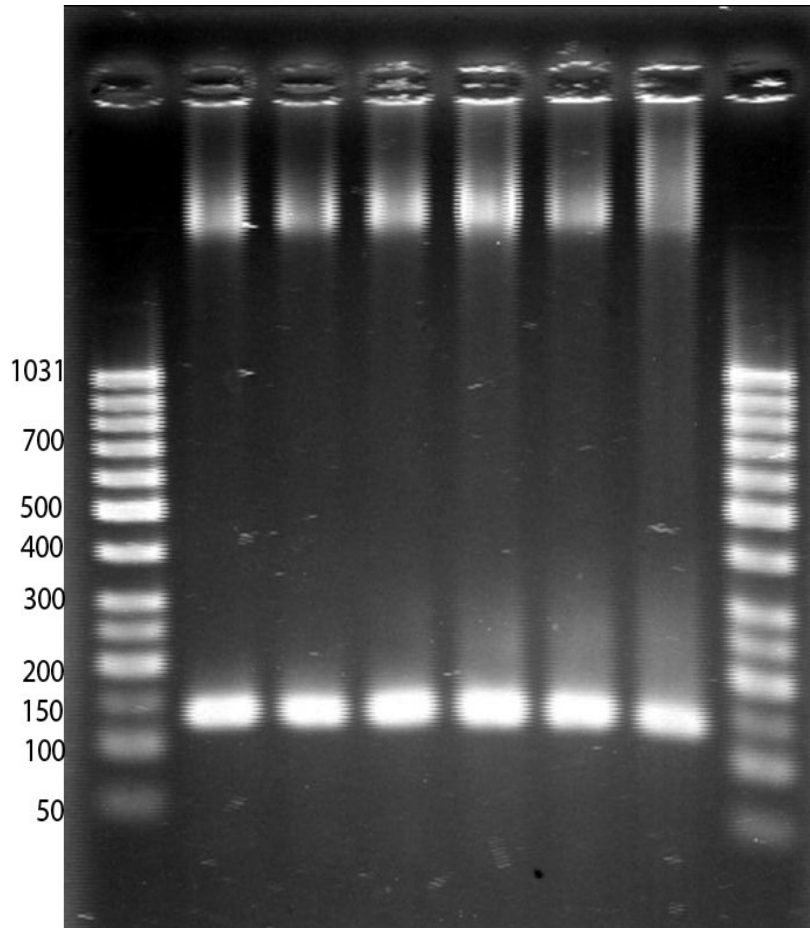
Process of Pertussis Detection at NIH



Within 1-2 days !!



PCR for detection of *Bordetella pertussis* from nasopharyngeal swab



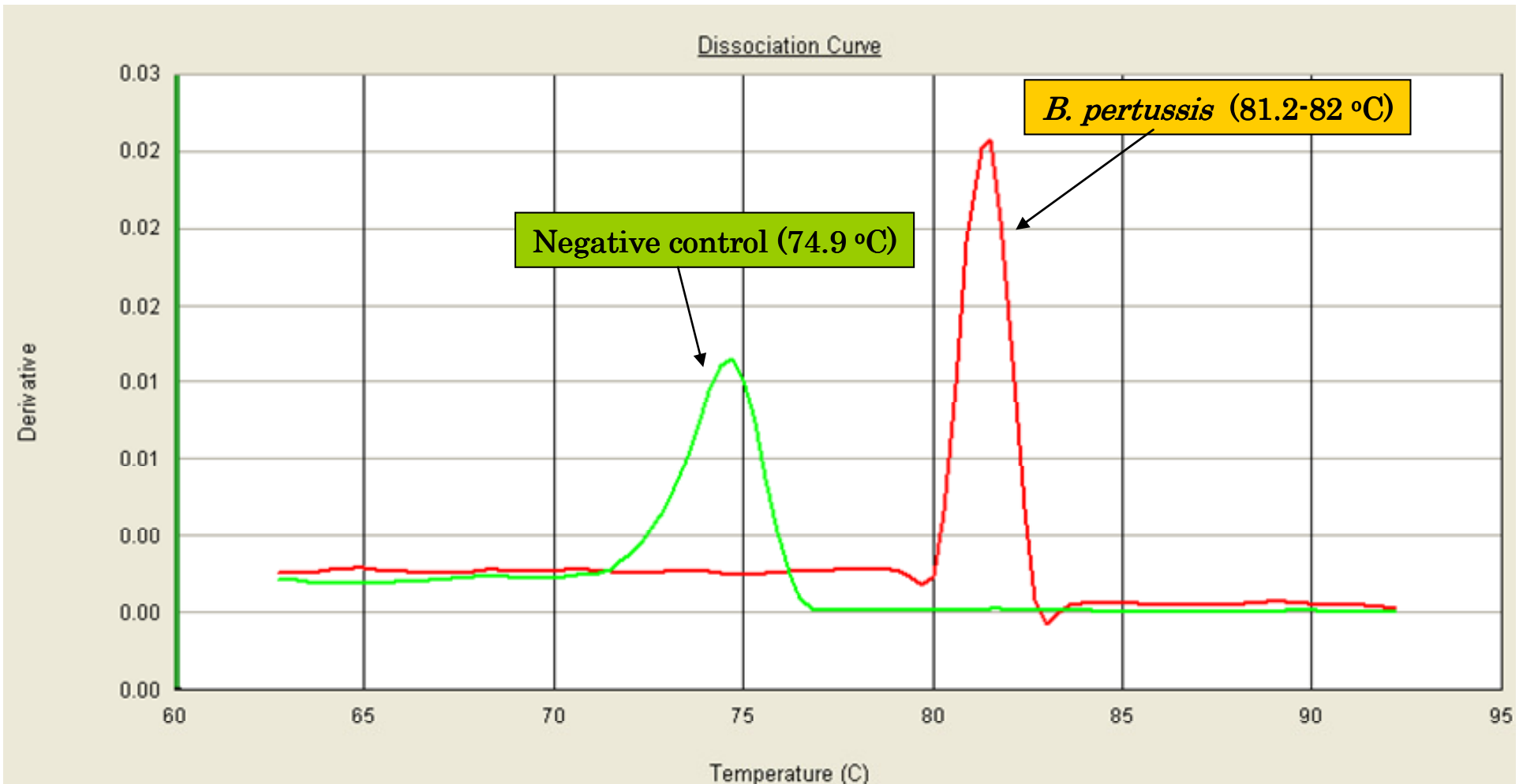
- Specificity = 97%

- Detection limit = 10^3 CFU/ml and 10^4 CFU/nasopharyngeal Swab or 100 - 1,000 CFU/assay

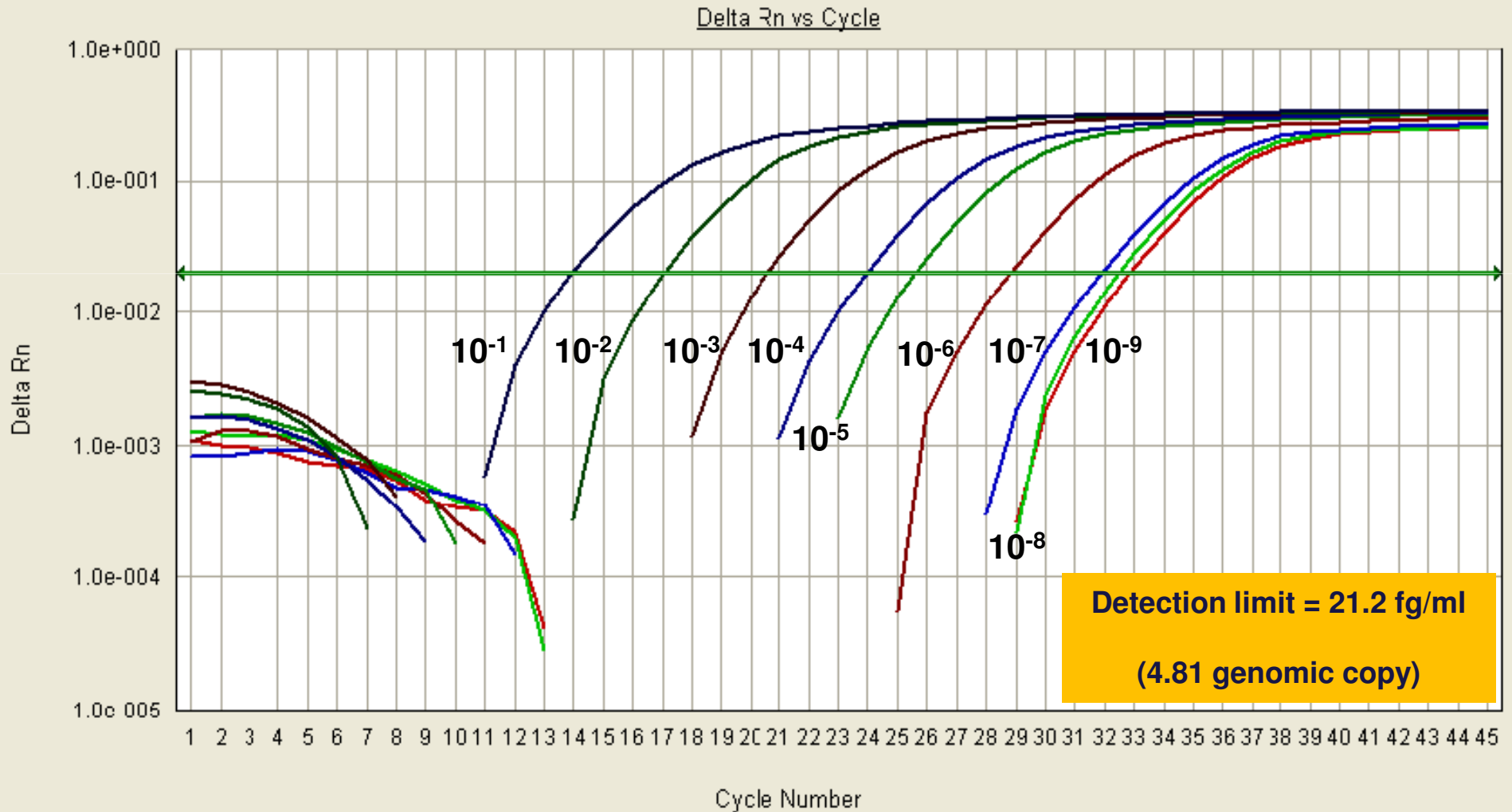
- Dracon swab is better than calcium alginate swab

- Storage of dracon swab for PCR: dried swab, < 3 days in room temperature

SYBR Green Real-time PCR for detection of *Bordetella pertussis*



Detection limit of SYBR green Real time PCR assay



Concordance of Assays

<i>IS481</i> SYBR green real-time PCR *	LAMP		Total	<i>Por</i> SYBR Green Real-time PCR**		Total	PCR		Total
	Positive	Negative		Positive	Negative		Positive	Negative	
Positive	98	9	107	93	14	107	69	38	107
Negative	12	99	111	0	111	111	0	111	111
Total	110	108	218	93	125	218	69	149	218

* *IS481* SYBR green real-time PCR is described by Ménard et al, 2007.

** *por* SYBR green real-time PCR is targeting on an upstream region of *B. pertussis* porin gene (our study).

Accuracy data	<i>Por</i> SYBR Green Real-time PCR (%)	LAMP (%)	Conventional PCR (%)
Sensitivity	86.9	91.6	64.5
Specificity	100	89.2	100
Positive predictive value	100	89.1	100
Negative predictive value	88.8	91.6	74.5

Multitarget Real-Time PCR (CDC method) Probe-based system

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2011, p. 4059–4066
0095-1137/11/\$12.00 doi:10.1128/JCM.00601-11
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Vol. 49, No. 12

Novel Multitarget Real-Time PCR Assay for Rapid Detection of *Bordetella* Species in Clinical Specimens[∇]

Kathleen M. Tatti,* Kansas N. Sparks, Kathryn O. Boney, and Maria Lucia Tondella

*Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases, Division of
Bacterial Diseases, Meningitis and Vaccine Preventable Diseases Branch, Atlanta, Georgia 30333*

Multitarget real-time PCR

CDC algorithm

Species	Multiplex			
	<i>ptxS1</i>	IS481	hIS1001	pIS1001
<i>B. pertussis</i>	+	+	-	-
<i>B. parapertussis</i>	+	-	-	+
<i>B. pertussis</i> and <i>B. parapertussis</i>	+	+	-	+
<i>B. holmesii</i>	-	+	+	-

Detection limit of multitarget real-time PCR

Genomic equivalents	<i>Bordetella pertussis</i> ptxS1 Ct values	<i>Bordetella pertussis</i> IS481 Ct values	<i>Bordetella parapertussis</i> IS1001 Ct values	<i>Bordetella holmesii</i> hIS1001 Ct values
1000	29	19	22	25
100	32	23	26	29
10	35	26	30	33
1	39	30	33	38
0.1	Negative	33	36	Negative

Summary

- PCR is an attractive tool for diagnosis of pertussis especially in the setting of the current resurgence of pertussis disease.
- PCR can provide timely results with improved sensitivity over culture.
- Careful specimen collection and transport and a general understanding of the PCR assays performed will better ensure that clinicians obtain diagnostic test results that reliably inform patient diagnosis.



Thank you very much