Advances in Pathogen Detection and Diagnostic Tools for Infectious Diseases

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The Future of Microbiology

The Future…
(can machine replace human being?)

Why do we see changes today?

- Traditional microbiology diagnostics are too slow to guide empiric therapy
- With rapidly rising healthcare expenses, the need for accurate, rapid diagnostics that improves patient care is critical
- Scientific and technical advances are driving diagnostic opportunities never before imagined
- Delivery of healthcare is dramatically changing with consolidation of hospitals, need for point-of-care diagnostics etc.

Presentation Objectives

To discuss updates in diagnostic tests

To review the principles of proper collection of handling specimens

To highlight gaps related to pathogen detection and diagnostic tools

Use of ID Diagnostic Tests

- Detection of specific pathogens,
- Discovery of new pathogens
- Determining appropriate therapy
- Monitoring response to therapy
- Assessing prognosis
- Infection control
- Disease surveillance

How often do you visit your laboratory?

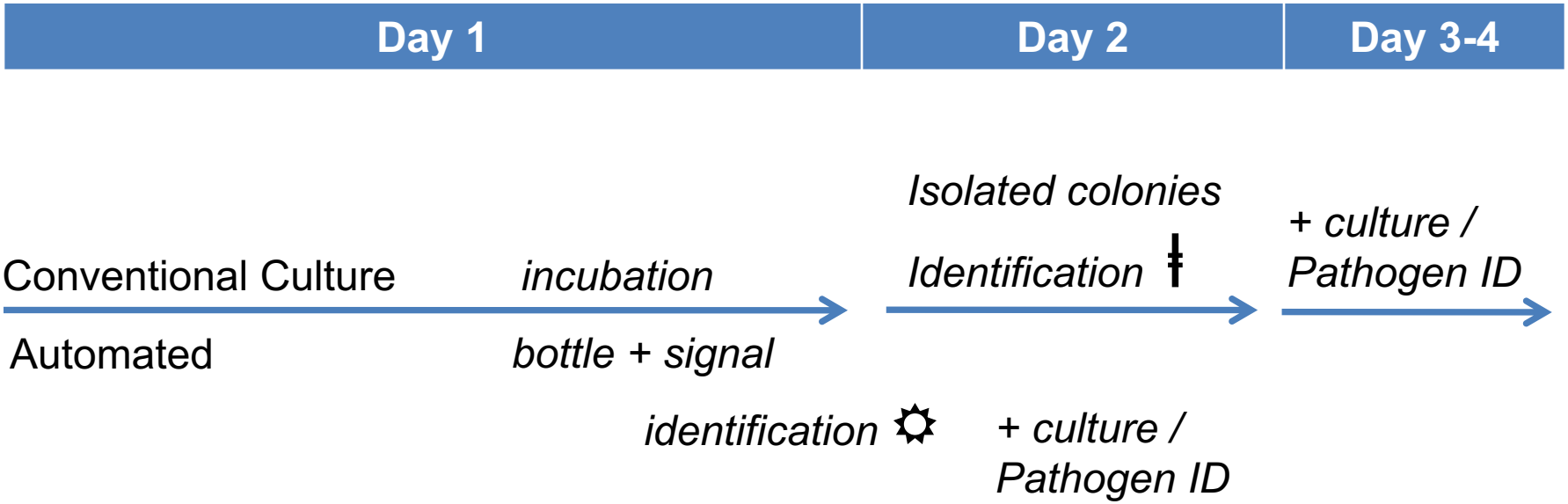
Current Diagnostic Methods Through Time

1860s:
Culture-based Tests

1980s-90s:
Antigen-based Tests (serological tests)

2000s:
Molecular Test Polymerase Chain Reaction (PCR) Tests

Conventional Method Challenged by New Technology (Automated System)



Conventional ID
(tube method)

Pathogen identified
after 24-48-72 hours

Semi-Automated ID
API (Analytical Profile Index)

Pathogen identified
after 16-18 hours

Today's Molecular Diagnostics

Real-Time PCR Detection Systems

- Rapid detection and identification of pathogens
- Do not provide antibiotic susceptibility information, ongoing development on genetic determinants of resistance however, extremely complex
- Will only detect a subset of possible pathogens hence
- Serve as an adjunct to standard of care

Today's Molecular Diagnostics

GeneXpert

- Xpert MTB/Rif test is recommended than conventional microscopy and culture as initial diagnostic test in children suspected of having MDR TB or HIV-associated TB (WHO Guidance for national TB program in children 2013)
- Xpert MTB/Rif test may be used rather than conventional microscopy and culture as the initial test in all children suspected of having TB (WHO Guidance for national TB program in children 2013)
- Can be used for testing of non-respiratory specimens CSF, lymph nodes and other tissues from children suspected of having extrapulmonary TB

26 Xpert MTB/Rif test sites established and functional in the country

- Provide results from unprocessed sputum samples in less than 2 hours
- Cost effective in high and even low prevalence populations
- A one negative result is equivalent to 3 negative smears

	Mean price for Xpert MTB/RIF	Range	Private laboratories offering Xpert MTB/RIF (N)	Laboratories contacted for price information (N)
Kenya	$80·60	$51–$171	5	5
India				
IPAQT member laboratories	$30·26	Fixed Price	76	..
Rest of private sector	$52·82	$27·84–$86·55	60	13
Pakistan	$37·26	$25·96–$58·65	4	4
Philippines	$155·44	$128–$183	11	9
Bangladesh	$74·75	$45·50–$130	4	4
Afghanistan	$50·00	..	1	1
Uganda	No Xpert	..	0	..
Vietnam	No Xpert	..	0	..
Indonesia	No Xpert	..	0	..
Myanmar	No Xpert	..	0	..
Nigeria	No Xpert	..	0	..
Cambodia	No Xpert	..	0	..

More than 50% of primary health-care visits were to a private health-care provider in the countries shown.[4] Prices correct at September, 2015.
IPAQT=Initiative for Promoting Access to Quality TB Tests.

Table: **Price paid by private patients for Xpert MTB/RIF in 12 high burden countries with high rates of private health-care use**

Molecular Diagnosis of Respiratory Tract Infections

- Fast replacing traditional tissue culture methods and serology in rapid identification of respiratory viruses (for many) and some bacteria
- Do not discriminate between infection and colonization
- Valuable test in immunocompromised host e.g. Rhinovirus infection or just persistent shedding?
- Rapid detection properly direct antiviral therapy

Current Diagnostic Methods and Time Required for Pathogen Identification

Diagnostic Method	Time for Pathogen Identification	
Gram stain	Minutes	
Culture (Conventional)	3-5 Days	
Culture (Automated)	1-2 Days	
Antimicrobial susceptibility	Days	
Acute and convalescent antibody	Days	
Antigen detection	Minutes to hours	
Polymerase chain reaction	1 to several hours	

Gram stain

- The most important staining procedure in microbiology
- Still the first line of diagnosis for infectious diseases, further development of molecular diagnostics will eventually make it obsolete, but for now they can be helpful
- Use gram stain results as your rapid diagnostic technique
- Important procedure for suitability criteria for culture

Suitability Criteria for Culture

Classification of sputum on the basis of leukocyte and squamous epithelial cell densities

Cell numbers per x 100 (low power) field

GROUP	LEUKOCYTE CELLS	EPITHELIAL
6	<25	<5
5	>25	<10
4	>25	10-25
3	>25	>25
2	10-25	>25
1	<10	>25

*Only sputum samples in categories 4-6 should be cultured.

Manual on ARI, WHO

The “well-chosen” sputum specimen

- Met suitability criteria
- Deep cough, grossly purulent
- *Best obtained before antibiotics*
- Transport in 1 to 2 hours

General Principles of Specimen Collection and Transport

Michael L. Wilson

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In this issue of Clinical Infectious Diseases, *we present the first article in a series entitled "Diagnostic Microbiology Updates." Although clinical microbiology is included in the curricula of virtually all infectious disease fellowships, the degree of emphasis on this subject varies considerably. Infectious disease physicians—even those who have direct responsibilities or consulting responsibilities for the microbiology laboratories of the institutions in which they practice—may be hard pressed to keep up with the rapidly changing content of the primary literature in clinical microbiology. The purpose of this series, therefore, is at least in part to fill this void and to provide concise updates for clinicians. The first article, written by Dr. Michael L. Wilson, reviews current concepts in specimen collection and transport. A key issue for all clinicians (which is not always sufficiently emphasized) is the quality of the specimen submitted to the laboratory. It is an axiom that if specimens of poor quality are submitted, the results generated by the laboratory will have little or no clinical utility. Dr. Wilson's article describes some of the methods available to assure that only specimens of good quality, i.e., those most likely to be useful clinically, are processed in the microbiology laboratory. Future articles will address specific types of specimens, groups of pathogens, and diagnostic techniques, including molecular methods. We hope this series will be informative and valuable to the readers of* Clinical Infectious Diseases, *and we look forward to your comments.*

Melvin P. Weinstein and L. Barth Reller

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Specimens submitted for microbiological testing require proper handling from the time of collection through all stages of transport, storage, and processing. Issues common to all clinical specimens submitted for microbiological testing include not only proper identification but also collection techniques that maximize recovery of microbial pathogens and minimize contamination. For specimens such as sputum and urine, the relative proportions of microorganisms present in vivo must be preserved, or culture results may be misleading. If specimens are handled properly, culture results are easier to interpret, patient care is improved, and costs are potentially decreased. Although most guidelines for specimen handling remain unchanged, a recent emphasis has been placed on modifying traditional practices to decrease or eliminate unnecessary work, increase laboratory efficiency, and make microbiological testing more cost effective.

Guide to proper specimen collection

Disease	Tests	Appropriate Specimen	Time of collection	Quantity	Turn-around time
• Acute bloody diarrhea • Cholera	Culture	Fresh stool	During active diarrhea	2-5 ml (liquid) 5 g (solid) pea sized	3-5 days
		Rectal swabs		1-2 swab	3-5 days
• Typhoid	Culture	Fresh stool	2nd to 3rd week after onset of illness	5 g (solid) pea sized	Minimum 5 days
		Rectal swab	2nd to 3rd week after onset of illness	2 swabs	3-5 days
		Blood	1st week after onset of illness	1:5 to 1:10 ratio with BCB	7 days

Guide to proper specimen collection

Disease	Tests	Appropriate Specimen	Time of collection	Quantity	Turn-around time
• **Leptospirosis**	Culture	Whole blood	Within 10 days of illness	3-5 ml	6 weeks
		CSF	Within 10 days of illness	0.5-2 ml	6 weeks
		URINE	2nd up to 30 days after onset of Sx	15-50 ml	6 weeks
	PCR	Same as above	Same as above	Same as above	3-5 days

Guide to proper specimen collection

Disease	Tests	Appropriate Specimen	Time of collection	Quantity	Turn-around time
• **Leptospirosis**	MAT	Serum	5-10 days or later after onset of Sx or after collection of acute serum	>1 ml	
	MAT	Serum	14 days after onset of Sx (single serum collection)	>1 ml	

Guide to proper specimen collection

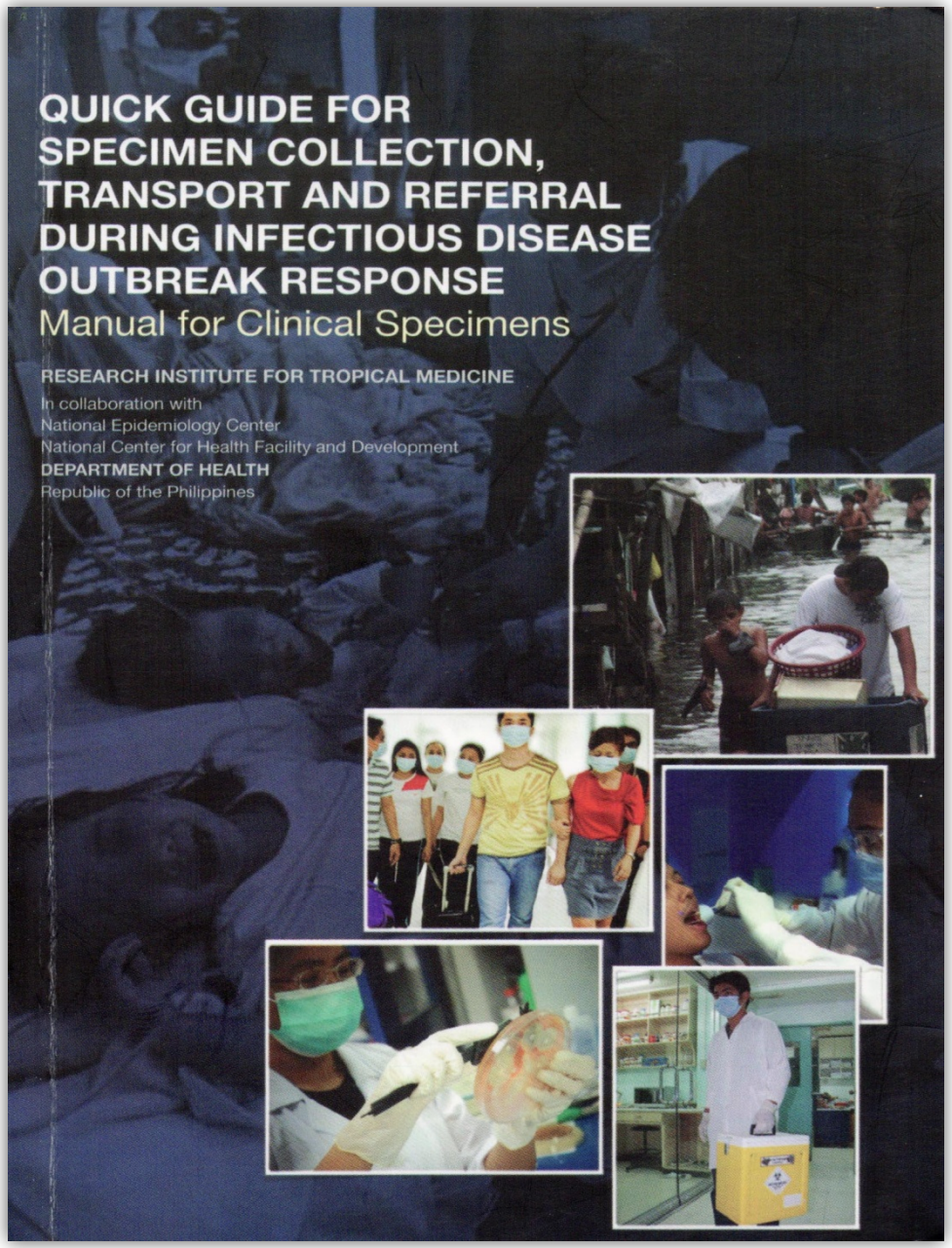
Disease	Tests	Appropriate Specimen	Time of collection	Quantity	Turn-around time
• Invasive meningococcal disease	Culture	Blood or CSF	Onset of illness	1:5 to 1:10 ratio with BCB	7 days
	PCR	Blood	Onset of illness	3-5 ml	3 days
		CSF	Onset of illness	0.5 to 1 ml	3 days
• Diphtheria	Culture	Throat & nasal swab, skin lesion	Onset of illness	2 swabs: 1 throat, 1 nasal	3-5 days
	PCR	Isolate	Onset of illness		3-5 days

Guide to proper specimen collection

Disease	Tests	Appropriate Specimen	Time of collection	Quantity	Turn-around time
• Pertussis	Culture	Nasopharyngial swab	<2 weeks post-cough onset	2 Dacron swabs L and R nostrils	8 days
		Nasopharyngial aspirate	<2 weeks post-cough onset	≥0.5 ml	8 days
	PCR	Nasopharyngial swab	<4 weeks post-cough onset	2 Dacron swabs L and R nostrils	3-5 days
		Nasopharyngial aspirate	<4 weeks post-cough onset	≥0.5 ml	3-5 days

Guide to proper specimen collection

Disease	Tests	Appropriate Specimen	Time of collection	Quantity	Turn-around time
• Bacterial meningitis	Culture	Blood	Onset of illness	1:5 to 1:10 ratio with BCB	7 days
		CSF	Onset of illness	0.5-1 ml	3 days minimum
	PCR	Whole blood	Onset of illness	3-5 ml	3 days
		Serum or CSF	Onset of illness	≥0.5 -1 ml	3 days
	Serology	Serum or CSF	Onset of illness	0.5-1 ml	1 day
		Whole blood	Onset of illness	3-5 ml	1 day
	Serotype/ serogroup	Isolate			1-2 days



for Viral diseases,
Parasitic diseases
and
Special
Pathogens

National Reference Laboratory

- Antimicrobial Resistance Surveillance Reference Lab (ARSP)
- National Voluntary Blood Services
- Bacterial Enteric
- Emerging Infectious Diseases
- Mycology
- Invasive Bacterial Diseases
- Polio and Enteroviruses
- Measles and Rubella
- Dengue and Chikungunya
- Infuenza
- Rotavirus
- Japanese encephalitis
- Malaria
- TB

Etiology of Pneumonia by PCR

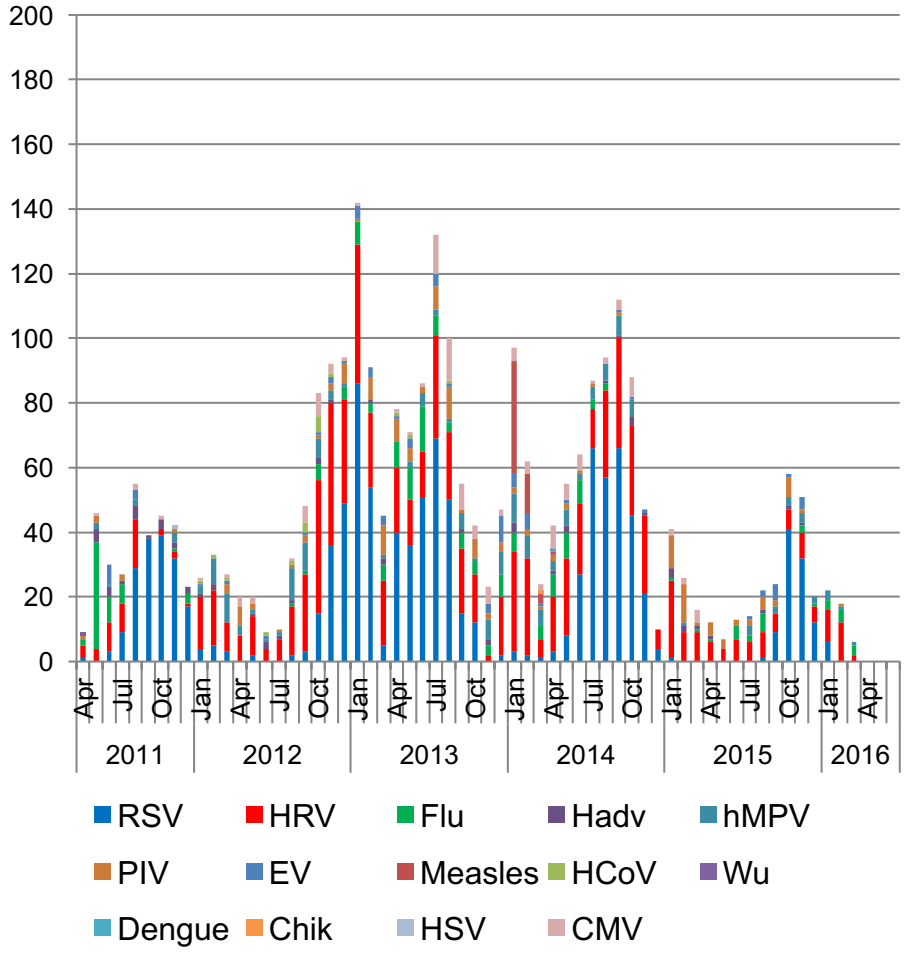
Pathogen	Chest Indrawing Pneumonia (CIP) (32%)	Very Severe Pneumonia (VSP) (68%)	Total (N=31)
Streptococcus pneumoniae	4	9	13
Hemophilus influenzae	3	2	5
Neisseria meningitidis	0	1	1
Methicillin Resistant Staphylococcus (MRSA)	1	0	1
Staphylococcus aureus	0	3	3
Pseudomonas aeruginosa	0	2	2
Klebsiella pneumoniae	0	2	2
Enterobacter aerogenes	1	0	1
Acinetobacter baumannii	1	2	3

Etiology of Atypical Pneumonia by PCR

Pathogen	Chest Indrawing Pneumonia (CIP)	Very Severe Pneumonia (VSP)	Total
Mycoplasma pneumoniae	0	3	3
Bordetella pertussis	3	18	21

Viral pathogens detected

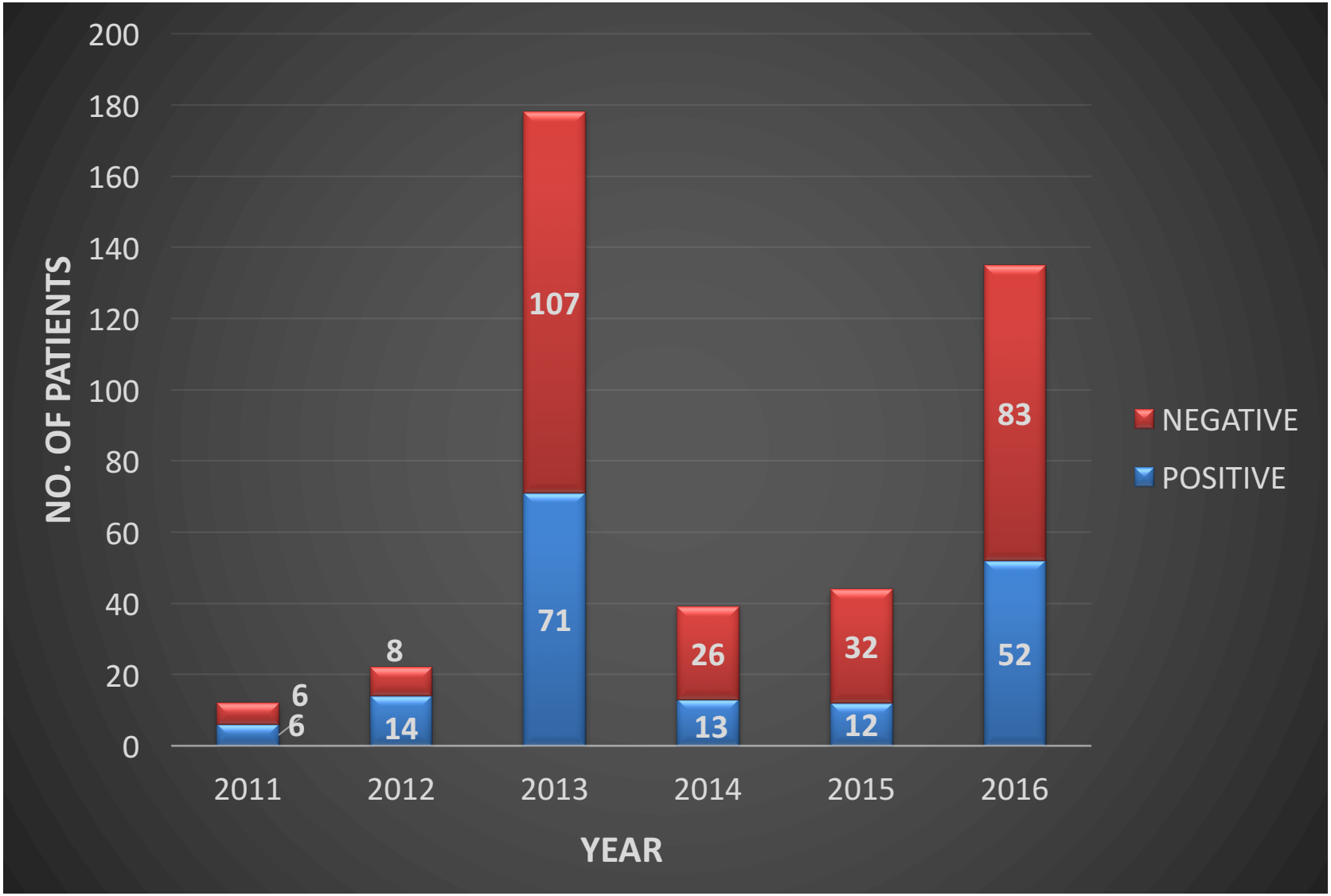
Hospital Sentinel Sites, 2011-2016



Virus	Frequency (%)
RSV	1026 (25)
HRV	736 (18)
Influenza	152 (3.7)
PIV	118 (2.9)
HAdV	41 (1.0)
hMPV	132 (3.2)
HEV	61 (1.5)
Measles	56 (1.4)
HCoV	13 (0.3)
Wu, HSV, Dengue, Chikungunya	1 each (0.03)
CMV	63 (1.5)
2 viruses	241 (5.9)
3 viruses	4 (0.1)
4 viruses	1 (0.02)
Negative	1406 (34.3)

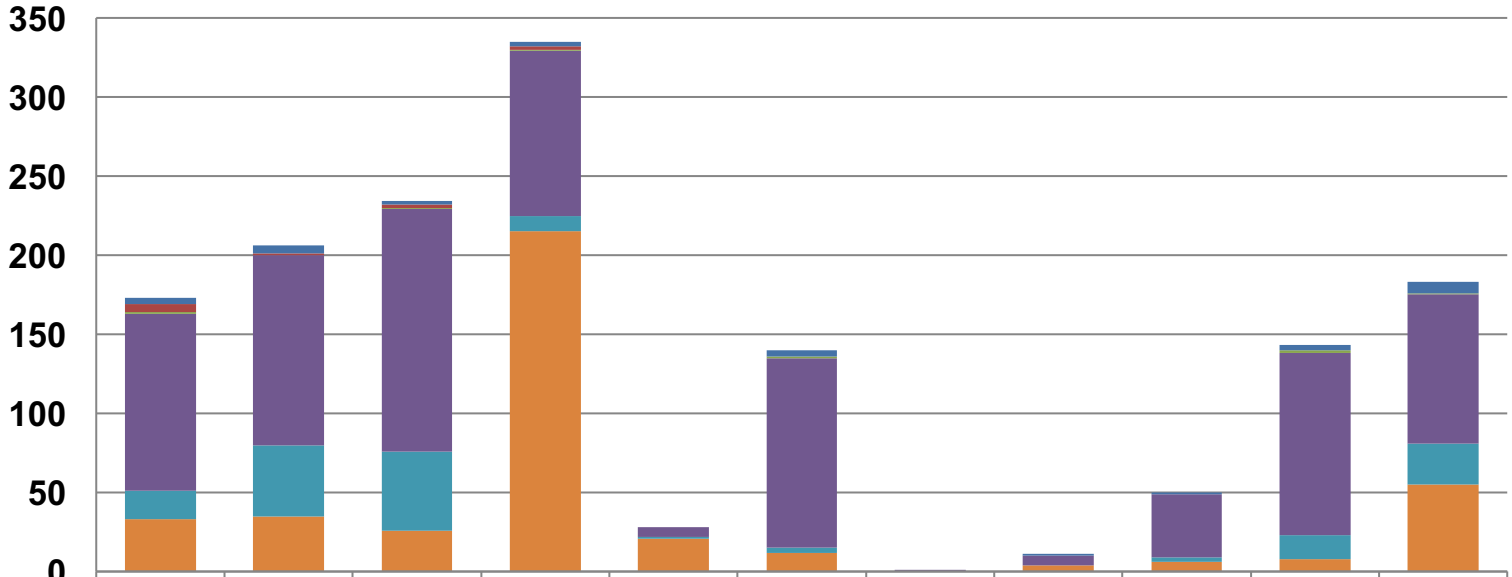
RITM-Tohoku Research Collaborating Center

Bordetella pertussis by PCR



Invasive bacterial pathogens

January – December 2016



	BGH MC	ITRM C	CVM C	JBLM RH	BMC	PCM C	PGH	SLH	WVM C	VSM MC	SPMC
Sp	4	5	2	3		4		1	1	3	7
Hi	5	1	2	2							
Nm	1		1	1		1				2	1
Negative	112	120	153	104	6	120	1	6	40	115	94
Inconclusive	18	45	50	10	1	3			3	15	26
Not done	33	35	26	215	21	12		4	6	8	55

Acute Meningitis Encephalitis Surveillance (AMES) - RITM-DOH-WHO

Serotypes of *S. Pneumoniae*

January - December 2016

Sentinel Hospital	Region	1	2	14	10A	12F/12A/ 12B/44/46	22F/22A	23F	6A/6B/6C /6D	Grand Total
BGHMC	CAR					1	1			2
ITRMC	1	2			1					4
CVMC	2					1				1
PCMC	NCR	2		1				1		4
VSMMC	7			1					1	2
SPMC	11		1							1
Grand Total		**4**	**1**	**2**	**1**	**2**	**1**	**1**	**1**	**14**

*Out of 29 positive samples, only14 were serotyped, the rest are for serotyping

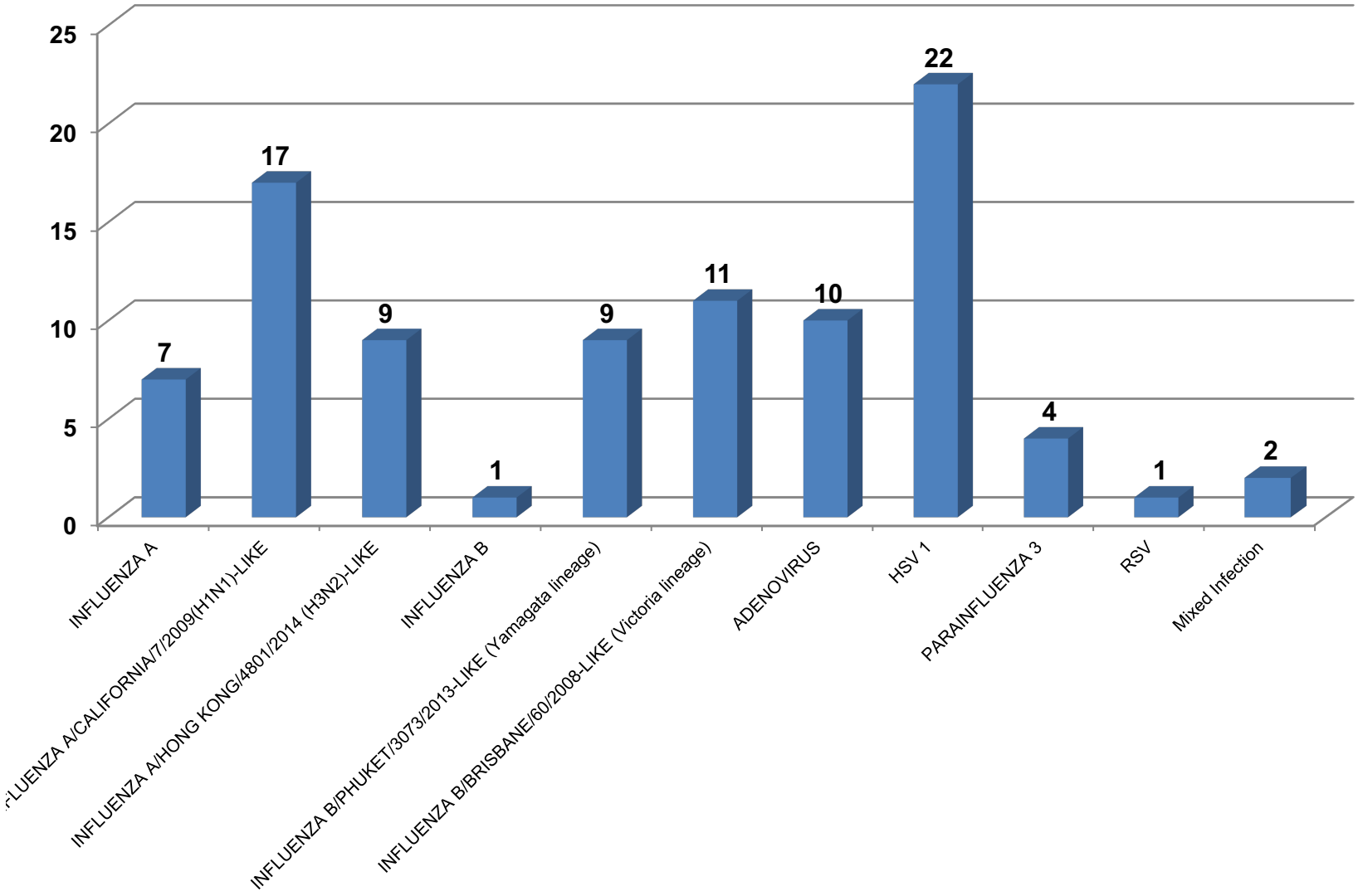
PCR

Quellung Reaction

Previous **Present**

Acute Meningitis Encephalitis Surveillance (AMES) - RITM-DOH-WHO

Viruses detected by Virus Isolation in the SARI Surveillance
January 1, 2016-December 31, 2016
N=784



Viruses detected by PCR in the SARI Surveillance January 1, 2016-December 31, 2016 N=784

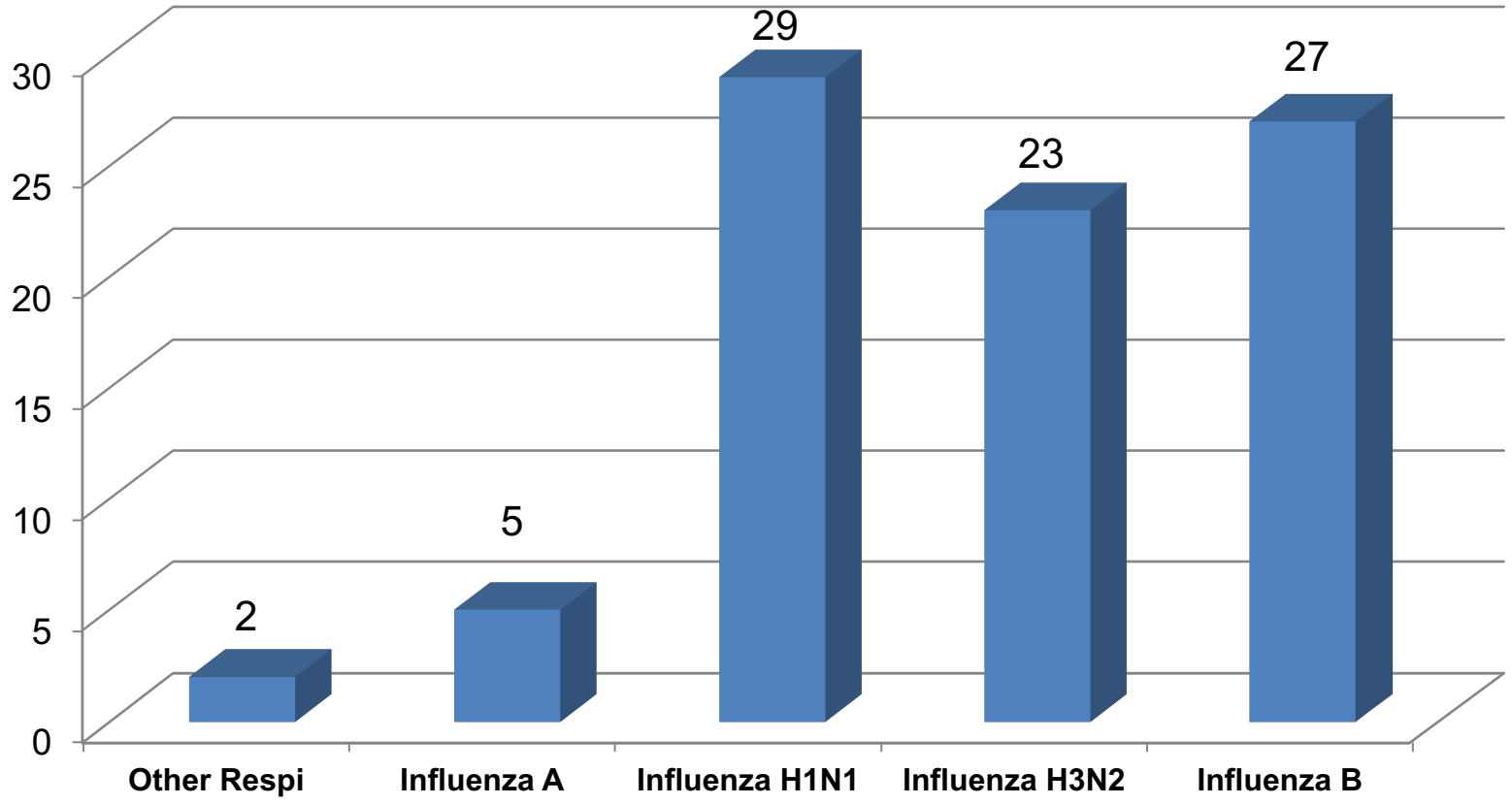
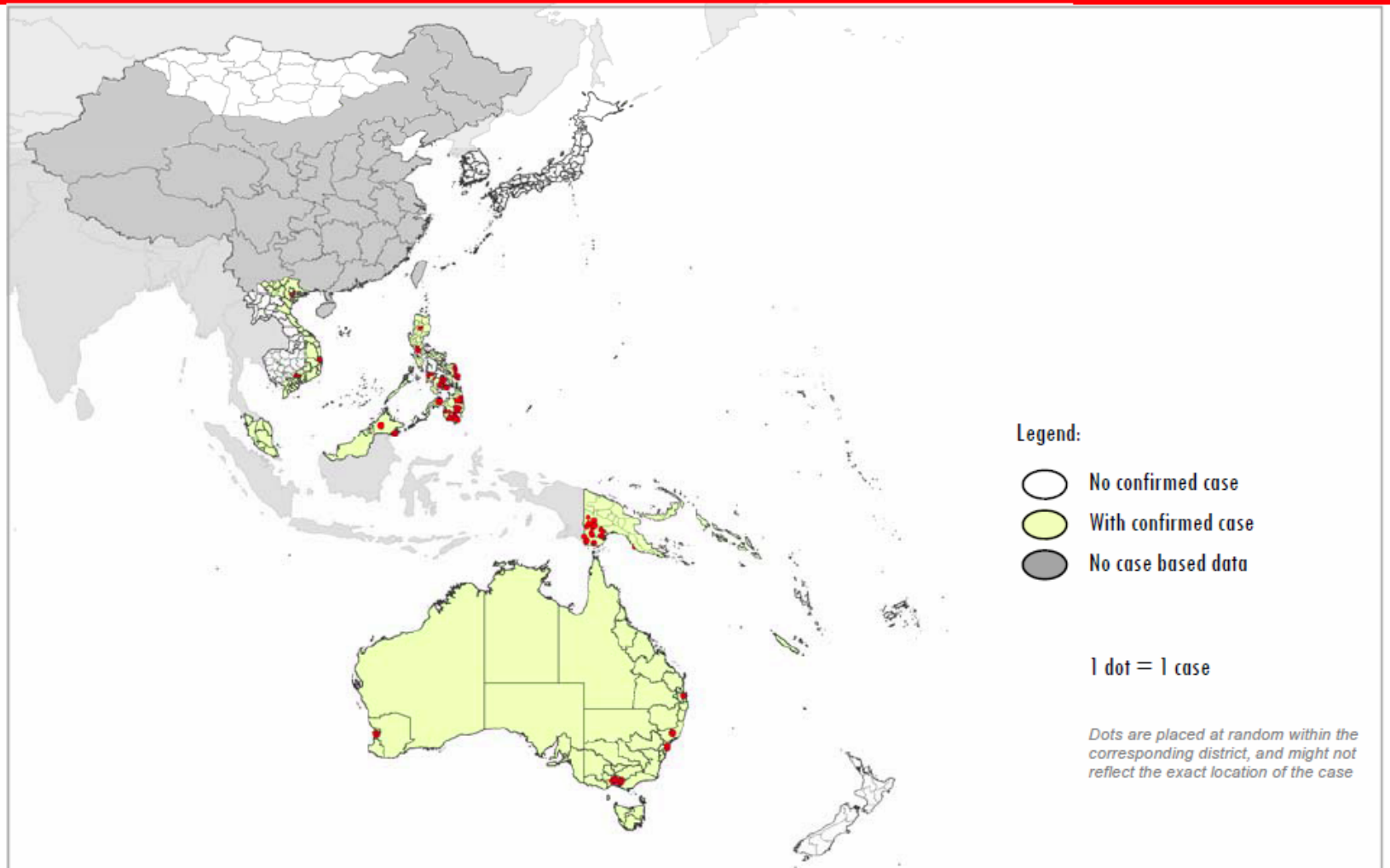
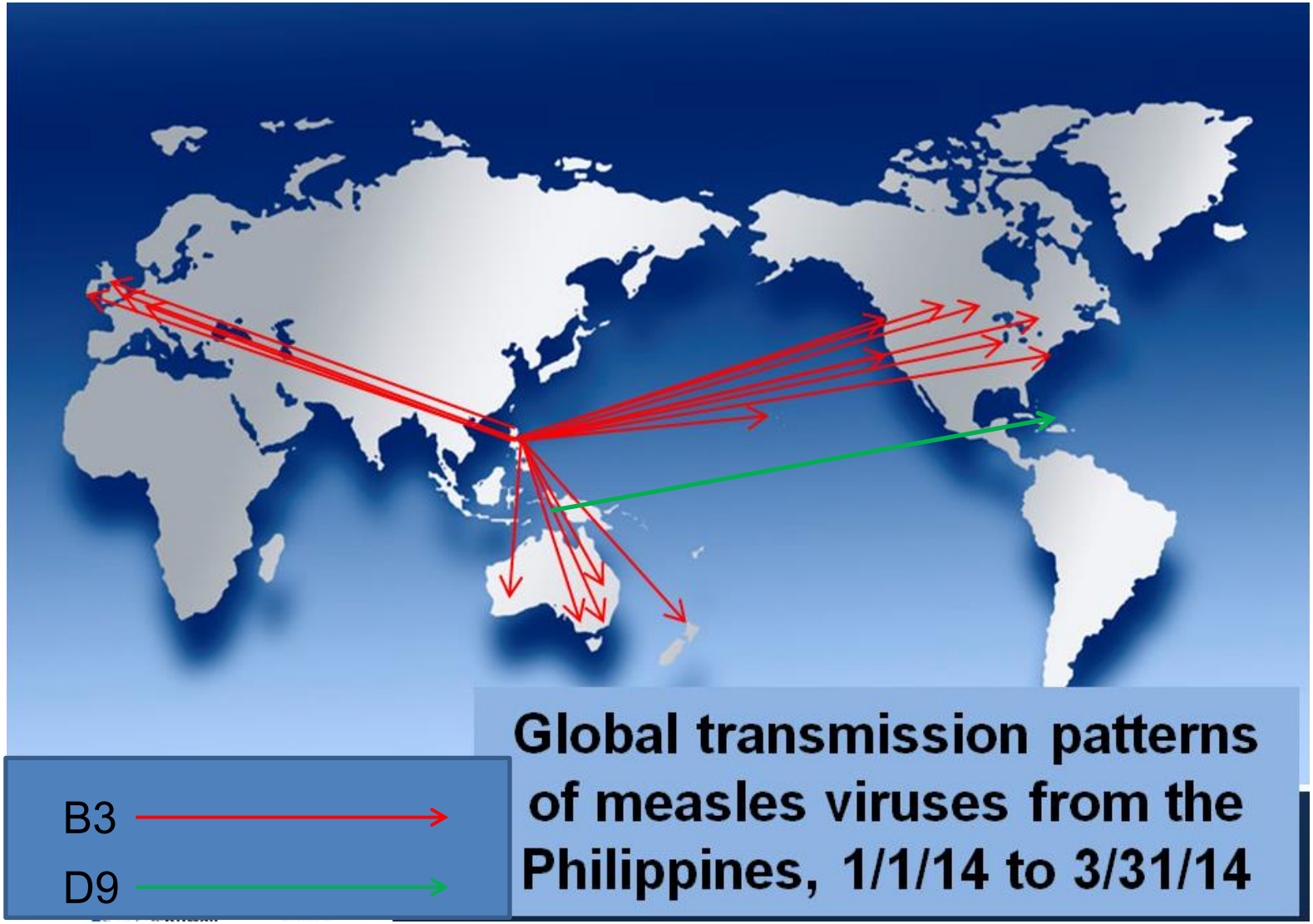


Figure 1. Distribution of confirmed measles cases with rash onset 1–31 January 2015, WHO Western Pacific Region



Source: Measles and rubella monthly country reports to the Western Pacific Regional Office by 20 February 2015


B3
D9
Global transmission patterns of measles viruses from the Philippines, 1/1/14 to 3/31/14

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Disneyland measles cases genetically similar to Philippines outbreak

by **Madison Park**, CNN

(CNN)—The measles cases linked to Disneyland are genetically similar to the one involved in a massive outbreak in the Philippines, according to an analysis.

The California outbreak likely started when a traveler who was infected overseas with measles visited the amusement park while infectious, according to the Centers for Disease Control and Prevention. But health officials don't know exactly who the source of the outbreak is.

Genetic analysis of the specimens from 30 California patients showed that the measles was of genotype B3, which is identical to the virus circulating in the Philippines. The CDC also cautioned that the same virus type has been found in 14 other countries.

The highly contagious disease has been damaging in the Philippines, infecting about 53,000 people and killing 110 people in 2014. The country has not seen outbreaks this year, although there have been a trickle of cases, said Dr. Julie Lyn Hall, the WHO Country Representative in the Philippines.

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Measles cases in Australia traced to Pinoy hip-hop dancer

January 6, 2014 2:37pm

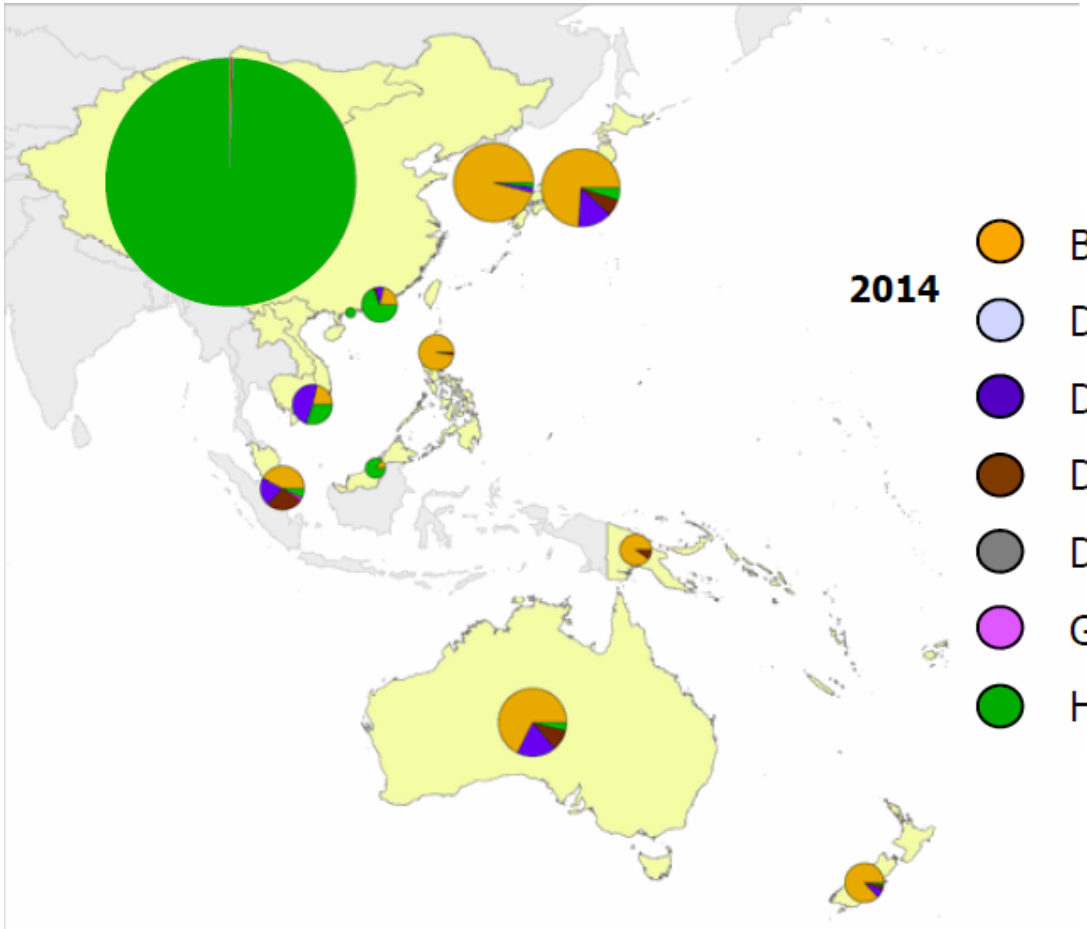
Health authorities in Australia confirmed last Friday that a Filipino has infected two other dancers during a recently concluded hip-hop competition in Sydney, New South Wales.

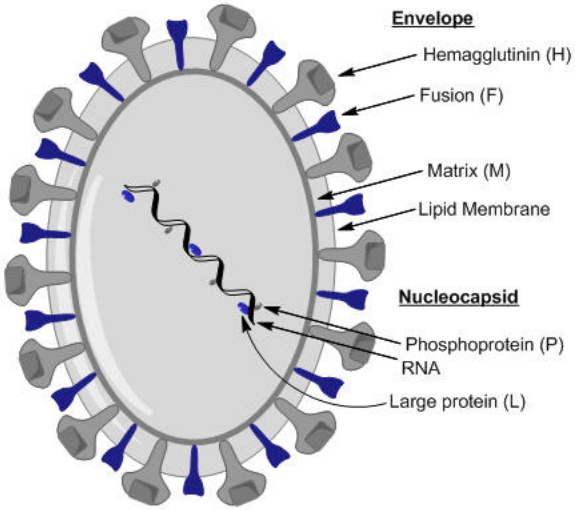
World Supremacy Battlegrounds (WSB) founder Marco Selorio said in a statement on January 3 that an adult male dance competitor from the Philippines began showing symptoms on the day of the competition, December 8.

But despite showing flu-like symptoms, the dancer participated in the event, apparently not knowing he had measles until his return to the Philippines on December 11.

The unnamed Filipino dancer is a member of FMD Xtreme, the champion of WSB 2013's open division.

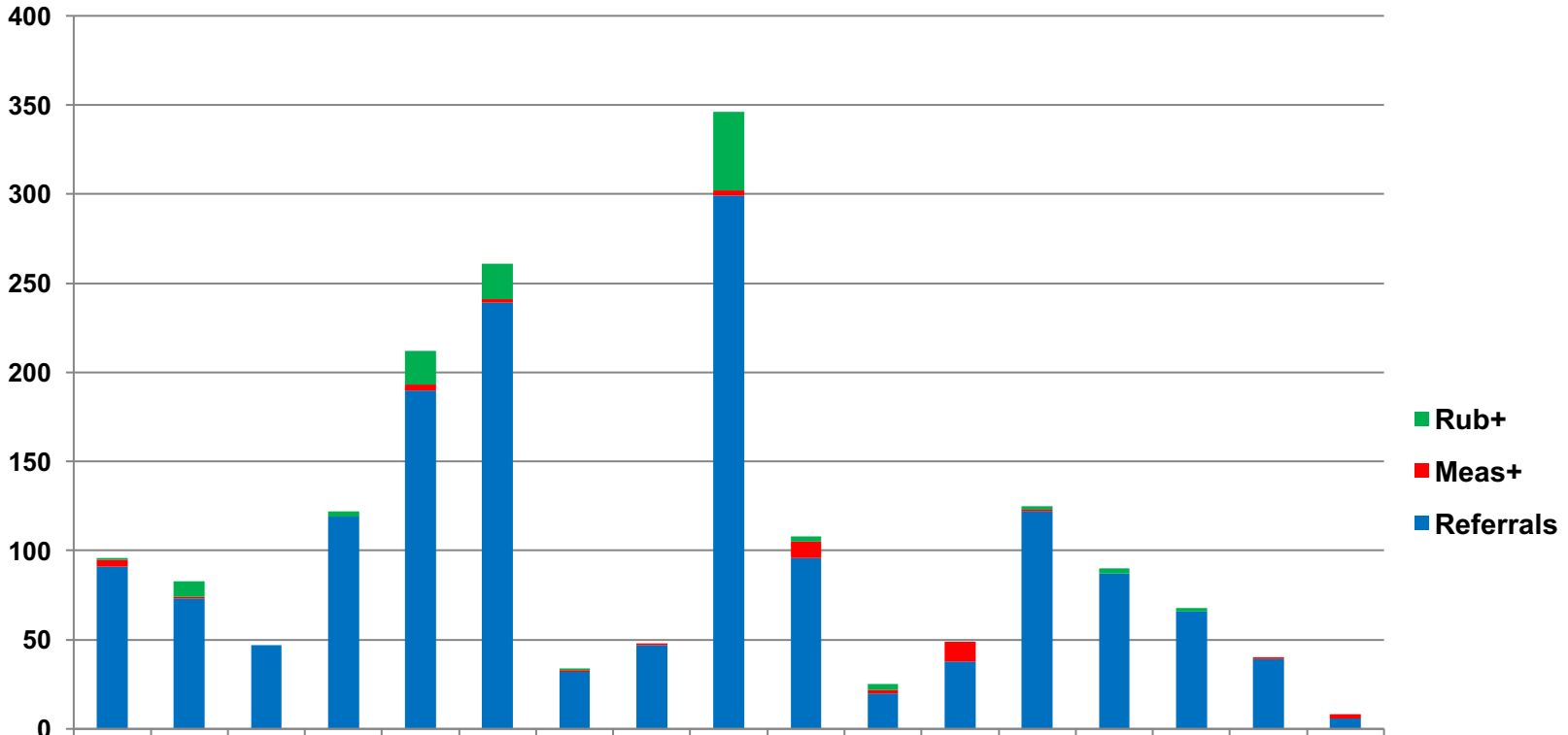
Measles Genotype Distribution of Cases in WPR, 2014





Measles Virus

Regional Distribution of Confirmed Measles and Rubella by IgM testing January to December 2016



	1	CAR	2	3	NCR	4A	4B	5	6	7	8	9	10	11	12	CAR AGA	ARM M
Rub+	1	9	0	3	19	20	1	0	44	3	3	0	2	3	2	0	0
Meas+	4	1	0	0	3	2	1	1	3	9	2	11	1	0	0	1	2
Referrals	91	73	47	119	190	239	32	47	299	96	20	38	122	87	66	39	6

An amazing technology?

Matrix Assisted Laser Desorption Ionization (MALDI-TOF)

- Next generation sequencing highly multiplexed assays, detect bacteria, virus, yeast, molds in a single test
- Looks at the protein signature of the bacteria and identification in a rapid manner
- Do not provide antibiotic susceptibility information

An amazing technology?

Matrix Assisted Laser Desorption Ionization (MALDI-TOF)

- Instrument very expensive, individual testing is inexpensive
- Requires expertise
- Dependent on the quality of database

Conclusion

- Technological advances have resulted in rapid identification and detection of pathogens and hold great promise for the future
- Conventional methods remain the dominant approach to diagnosing patients in the country
- The principles of patient selection, adequate and careful specimen collection, handling and transport, appropriate methods used and accurate result interpretation are the essentials in the effective care for our patients

A typical hospital lab in a decade or two?

Advancement in pathogen detection is being driven by clinicians having higher expectations for the laboratory. We want results fast in a time frame that will influence our decision making.

The next move is yours, critical to your clinical management, patient's health and outcome...

Thank you!