Measles/Rubella Serosurvey Experiences in Japan

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NIID
国立感染症研究所

NESVPD

- Originally started in1962
- Annually (depending on target diseases)
- To understand the actual situation of **herd immunity** or **prevalence of pathogens**, promoting effective management of immunization program
- Conducted by the Infectious Diseases Control Division, Ministry of Health, Labour and Welfare, Japan supported by prefectural health authorities and NIID

National Epidemiological Surveillance of Vaccine-Preventable Diseases (NESVPD)

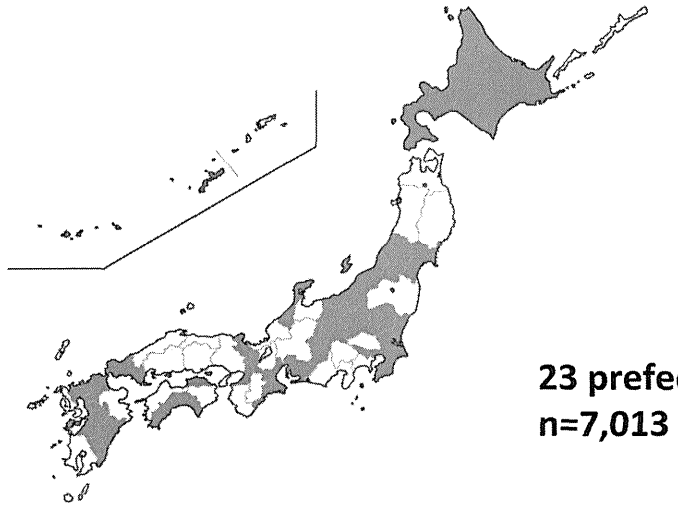
感染症
流行予測調査
NESVPD

Target diseases:

1. Poliomyelitis (Seroprevalence, pathogens)
2. Influenza (Seroprevalence, pathogens)
3. Japanese encephalitis (Seroprevalence of humans and pigs)
4. **Measles (Seroprevalence)**
5. **Rubella (Seroprevalence)**
6. Pertussis (Seroprevalence)
7. Diphtheria (Seroprevalence)
8. Tetanus (Seroprevalence)

Measles

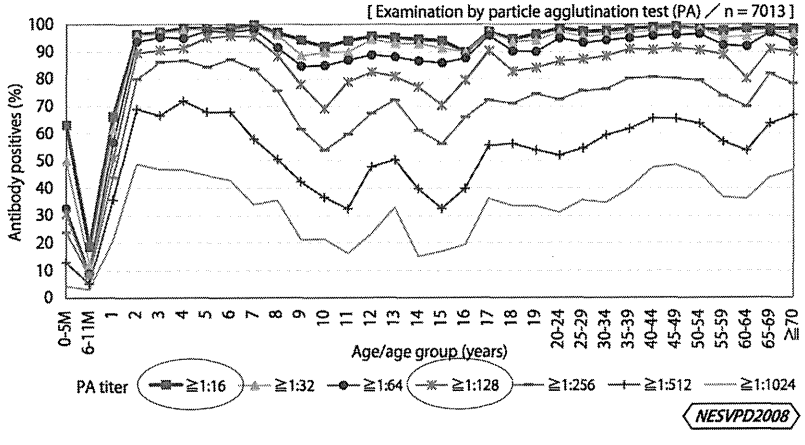
Sample collection for measles-seroprevalence survey in 2008



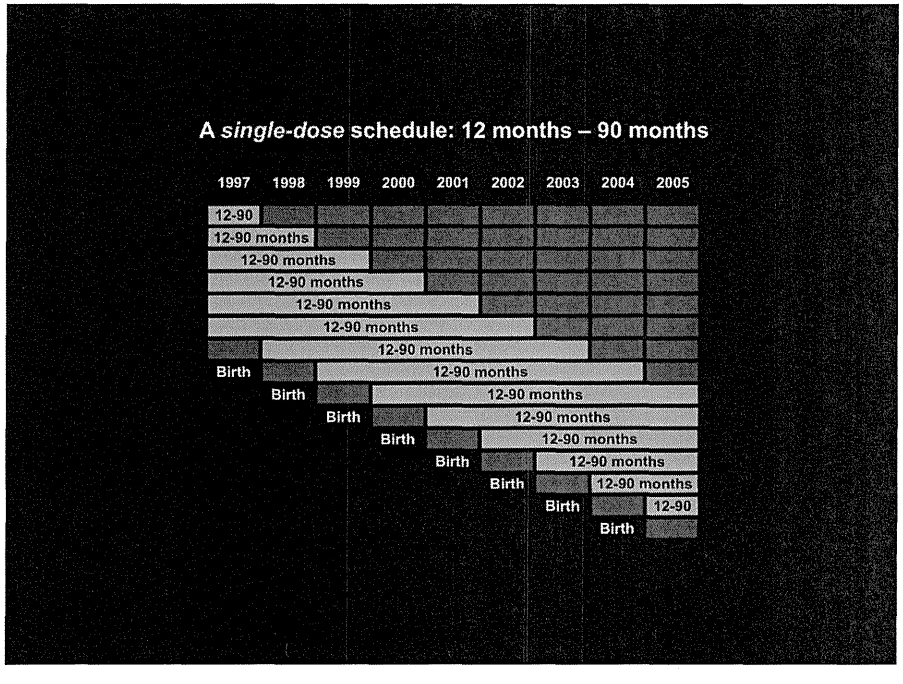
23 prefectures
n=7,013

Age distribution of measles PA antibody positives in Japan, 2008

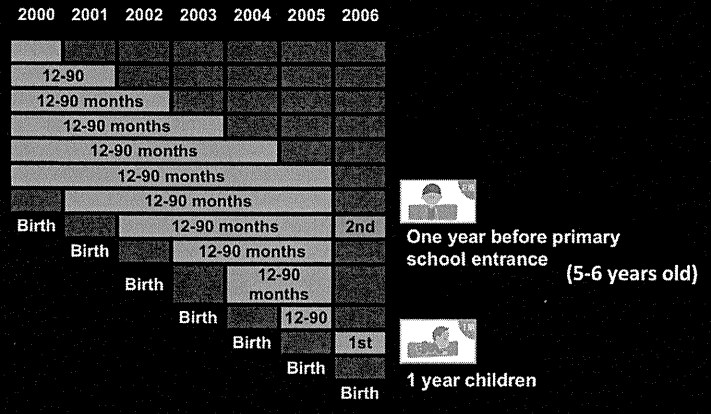
— National Epidemiological Surveillance of Vaccine-Preventable Diseases, 2008 —



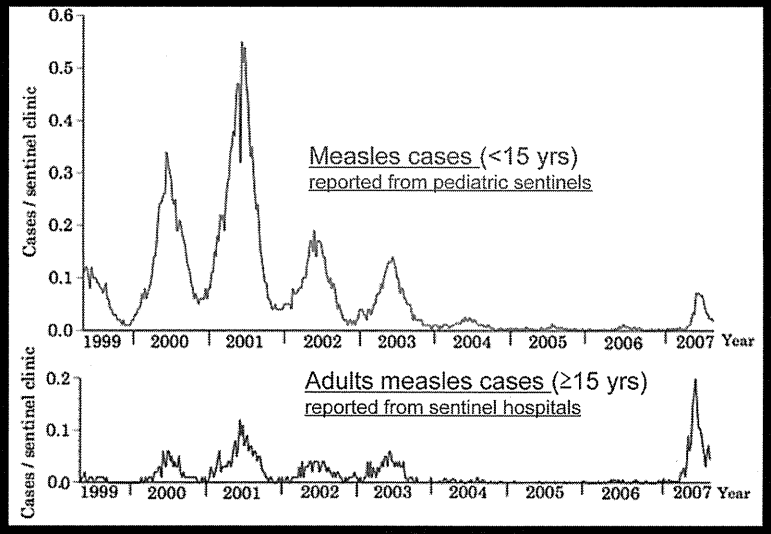
A *single-dose* schedule: 12 months – 90 months



2006 June
Introduction of the *two-dose* measles vaccination schedule

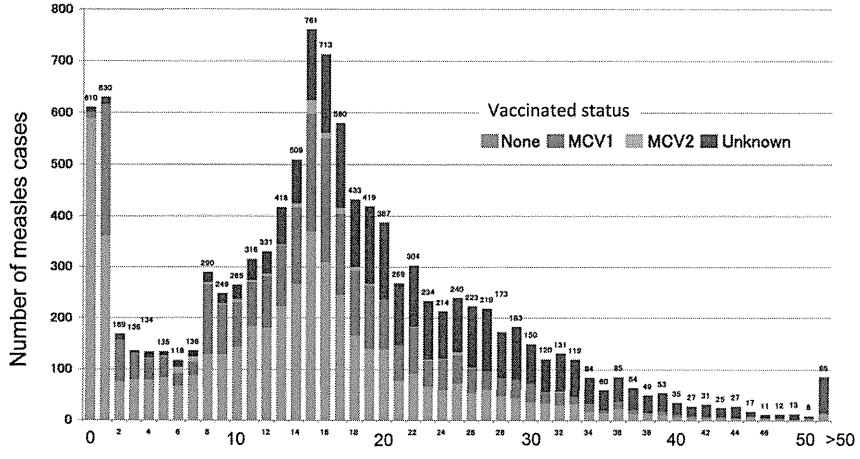


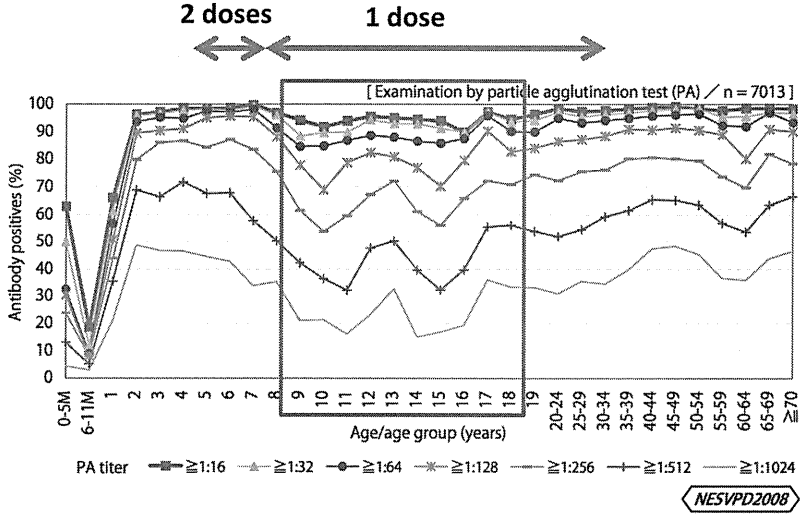
Weekly cases per sentinel from 14th week, 1999 to 31st week, 2007
Data from NESID, Japan



IASR Vol. 28 (No.331) September 2007

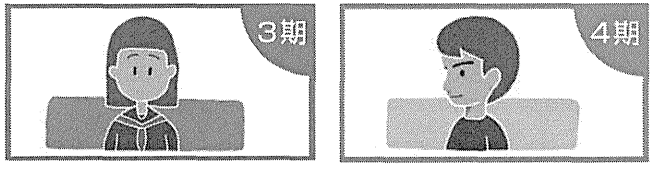
Cumulative measles cases by age and vaccinated status in 2008 (n=11,005)



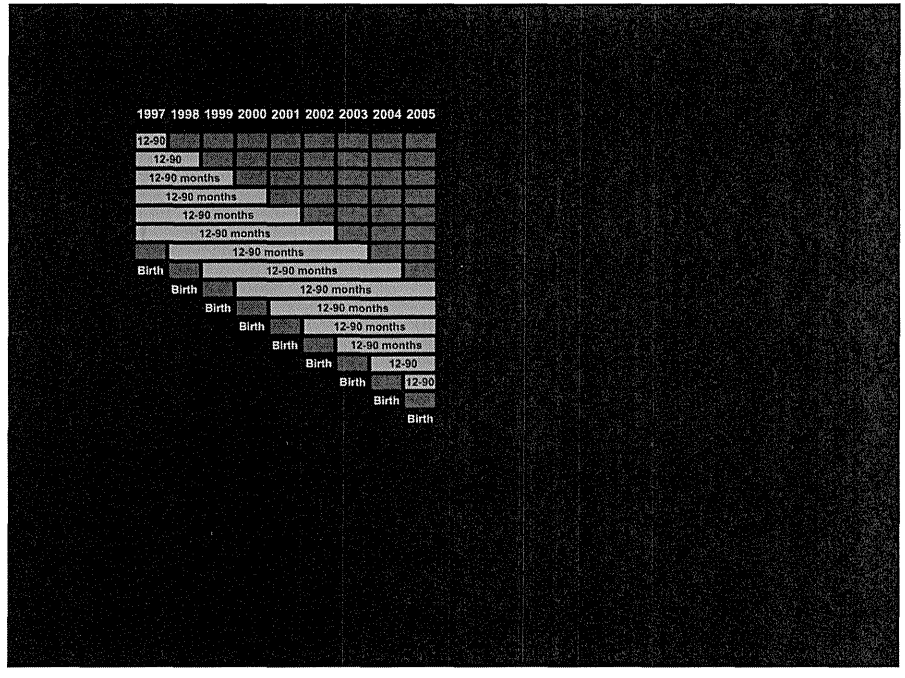


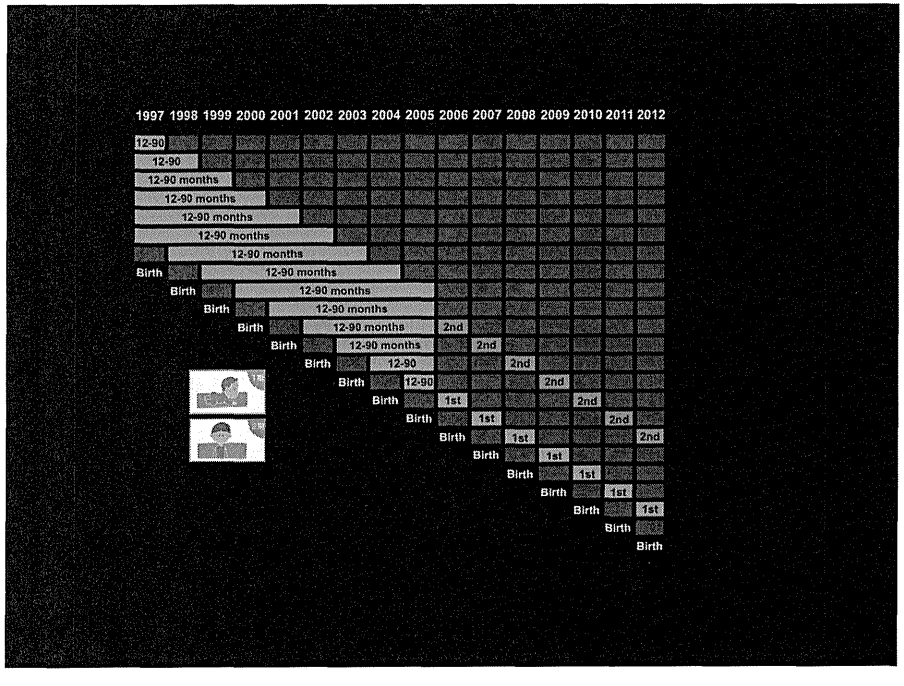
Dec 28, 2007
Special guidance for measles
by Ministry of Health, Labour and Welfare, Japan

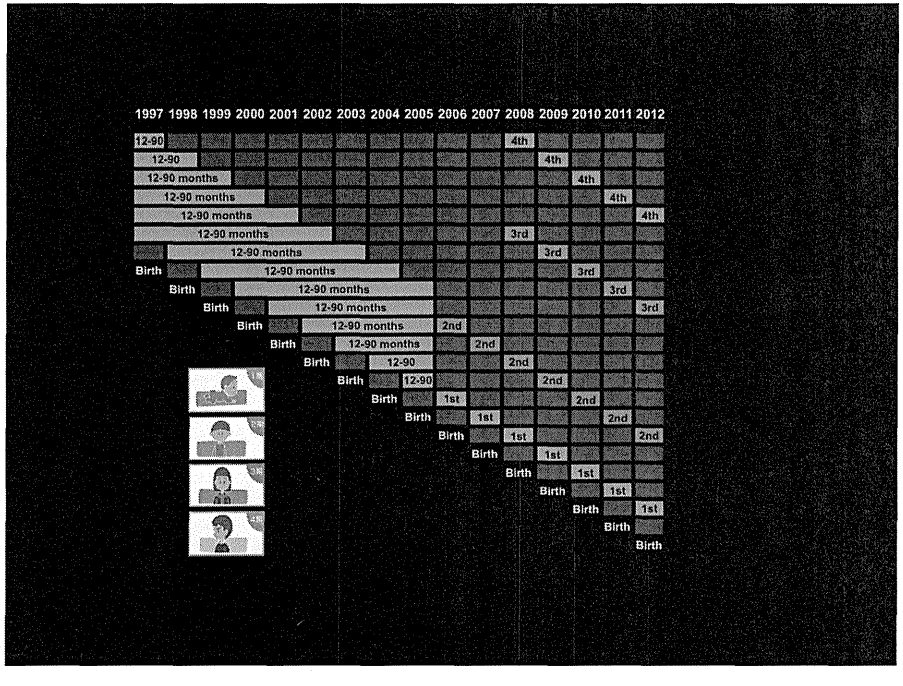
Supplementary immunization for 5 years targeting at teenagers (2008-2012)

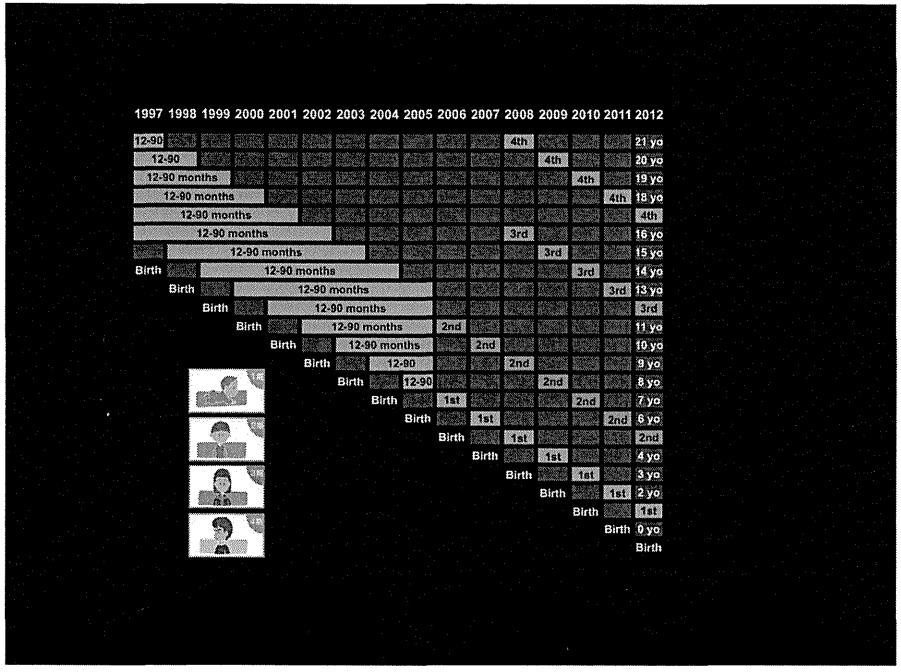


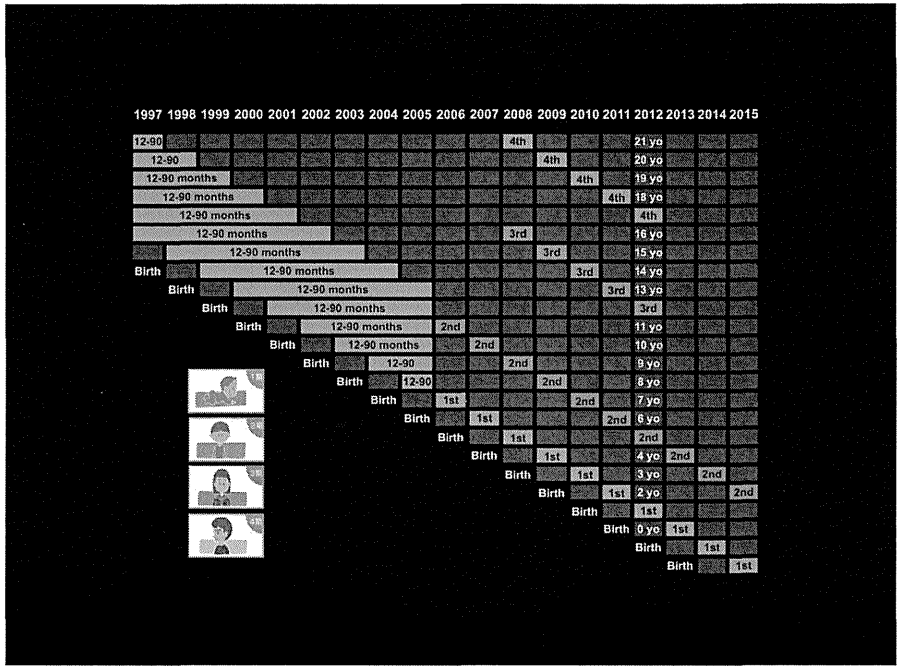
(3rd cohort) at 1st grade age of junior high school (7th graders)
(4th cohort) at 3rd grade age of high school (12th greders)







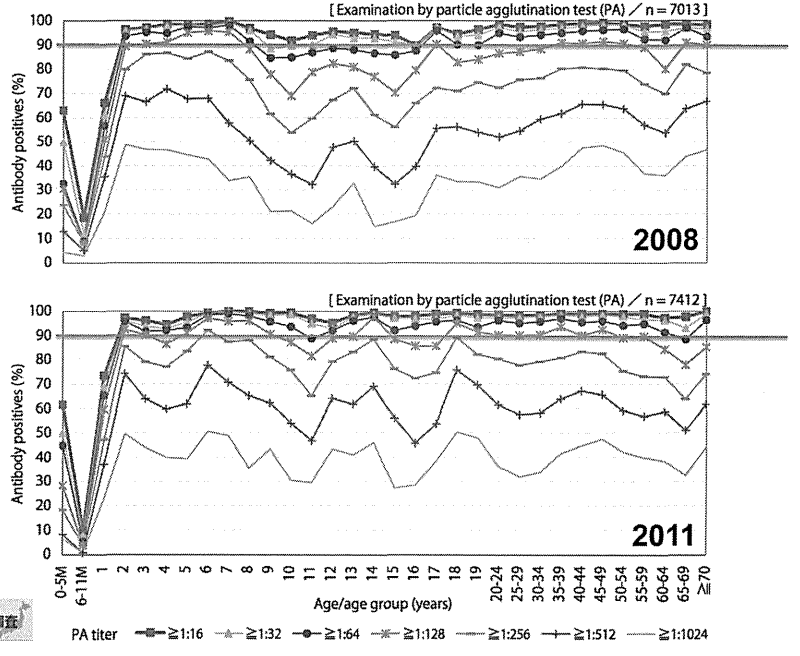


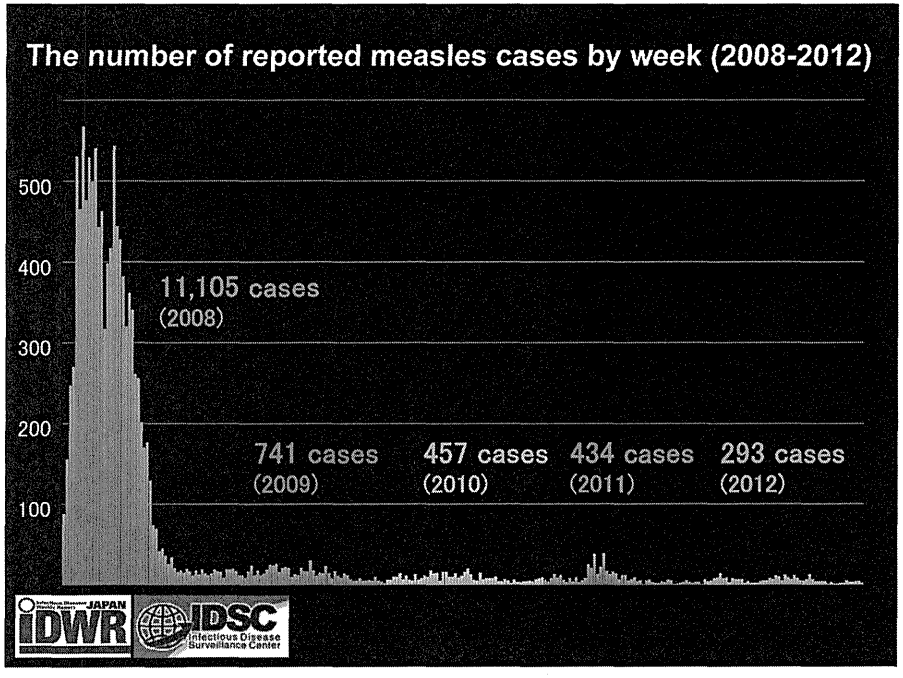


MCV (MR) immunization coverage

			FY 2008	FY 2009	FY 2010	FY 2011
1期	Routine vaccination programs	12 – 23 months	94.3%	93.6%	95.6%	95.3%
2期		5 – 6 years	91.8%	92.3%	92.2%	92.8%
3期	Catch-up vaccination programs	7th graders	85.1%	85.9%	87.2%	88.1%
4期		12th graders	77.3%	77.0%	78.8%	81.4%

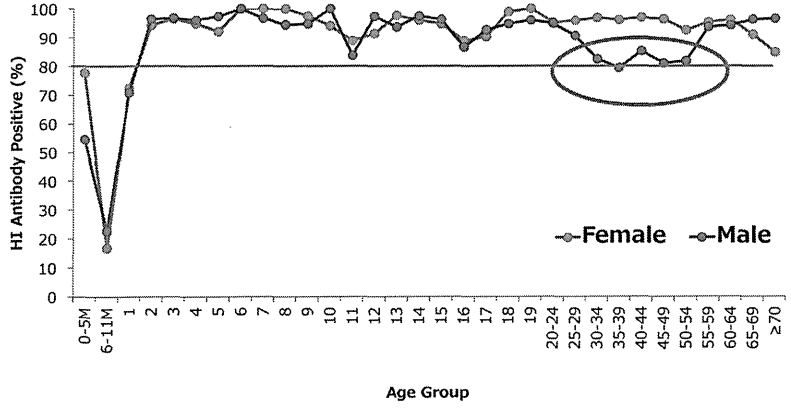
IDSC Infectious Disease Surveillance Center





Rubella

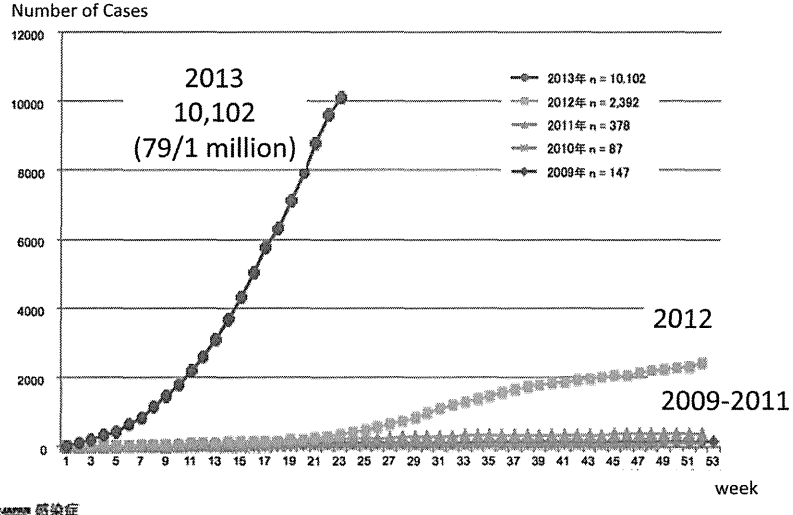
Age and sex distribution of rubella HI antibody positive (HI titer: ≥8) in 2011



Examination by hemagglutination inhibition (HI) test/n=2824(F), 2445(M)

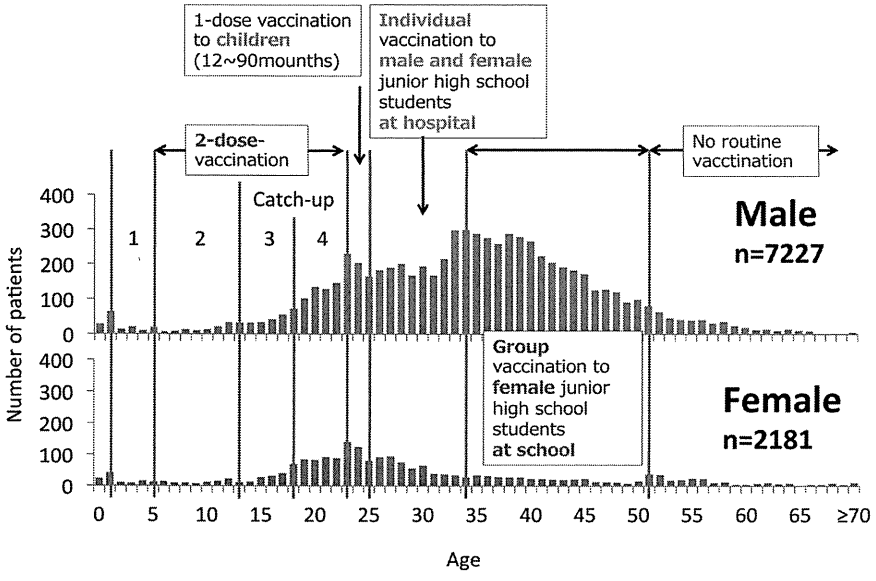
感染症流行予測調査 NESVPD

Cumulative rubella cases by week (2009～2013.23w)



IDWR 感染症発生動向調査週報

Rubella patients reported in 2013 (1-22w) in Japan



Historical transition of vaccination strategies for rubella control in Japan

- 1977 - 1994
  Group immunization for **female** junior-high school students **at school**
- 1995 - 2006
  Immunization to **children** (boys and girls) at 12-90 months
  + **Individual** immunization for **male and female** junior-high school students **at hospitals**
- 2006 – present
  Two-dose schedule of MR combined vaccines
  (1st cohort) 1 year children
  (2nd cohort) One year before primary school entrance
- 2008 - 2012
  Supplementary immunization for 5 years targeting at ages (teens)
  (3rd cohort) at 1st grade age of junior high school
  (4th cohort) at 3rd grade age of high school

Summary

- National surveillances for seroprevalence to Mealses and Rubella are conducted regularly in Japan.
- The data collected from this program are useful to indicate susceptible groups and should be used to promote effective management of immunization program.

CRS surveillance in Japan

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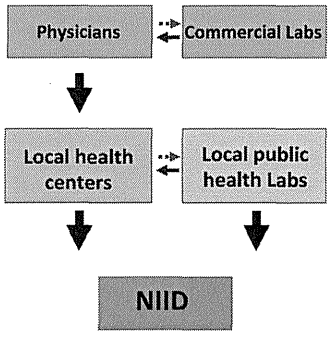
Surveillance system for rubella and CRS in Japan



Rubella

based on clinical or laboratory diagnosis

case-based reporting from 2008

CRS

Clinically- and laboratory-confirmed

case-based reporting from 1999

Criteria for CRS reporting in Japan

Clinical symptoms

based on criteria for "clinically confirmed CRS cases" defined by WHO

Laboratory diagnosis

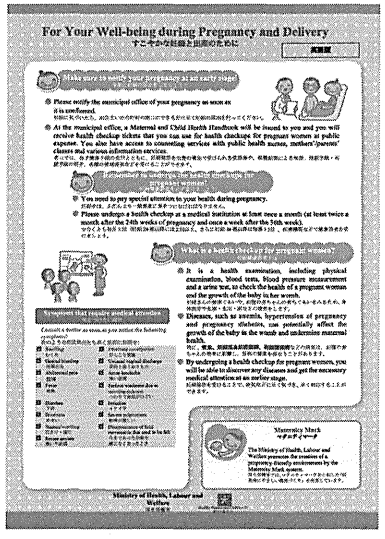
Serological testing or viral detection

Collection data

History of maternal rubella infection

Week of gestational age at rubella onset
Area of rubella infection
Vaccination history
etc.

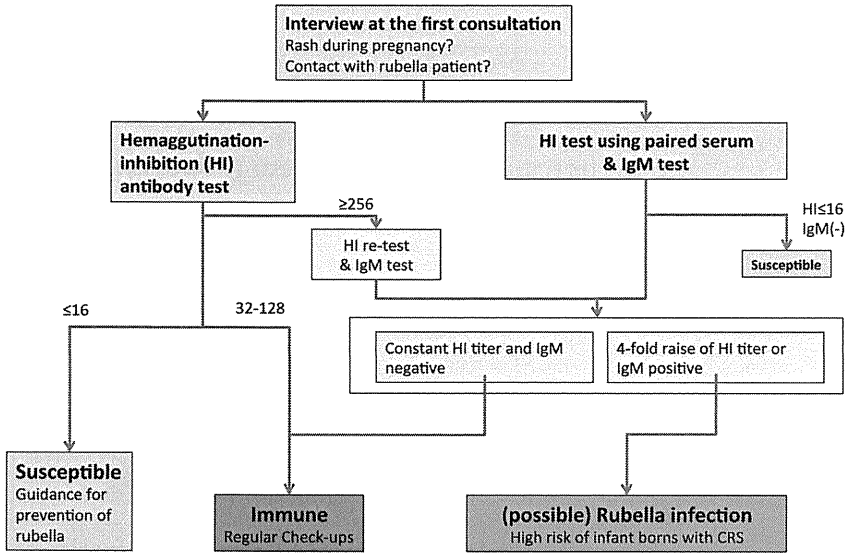
Public support for health checkup of pregnant women



Tickets for health checkups

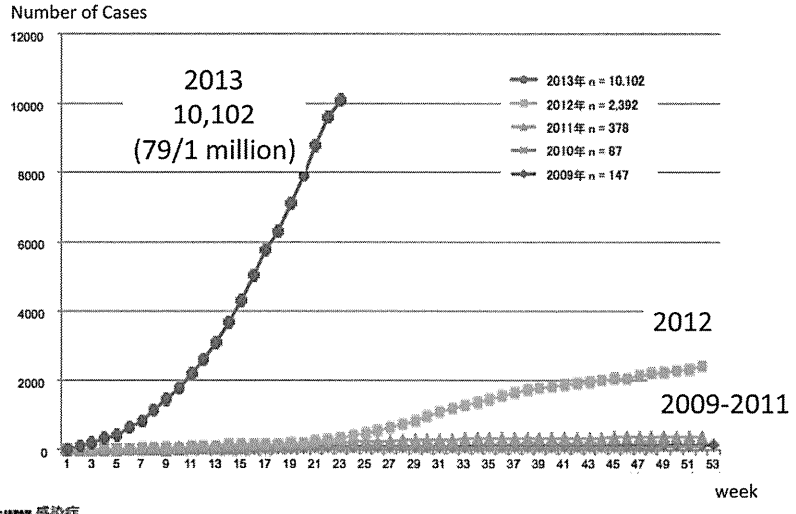
1. **Basic health checkups**
   - Blood testing including that for anti-Rubella antibody
2. **Medical checkups**
   - Biopsy for cervical cancer
   - Ultrasonography
   - Testing for group B Streptococcus
   - Testing for chamydia infection

Algorithm for evaluation of pregnant women with rubella in Japan



Based on Clinical guidelines for obstetrical practice in Japan (2011)
by the Japan Society of Obsterics and Gynecology and the Japan Association of Obstericians and Gynecologists

Cumulative rubella cases by week (2009～2013.23w)



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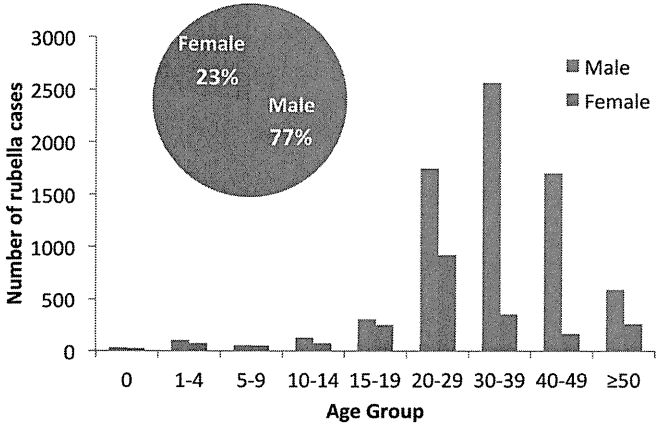
Age and sex distribution of rubella patients (2013.1-22w)



IDWR 感染症発生動向調査週報

Number of reported CRS cases (1999～2013.5)

Year	Number of CRS	Number per 1 million birth	comments
1999	0	0	1 dose-rubella vaccination at 12-90 month-old (since 1995)
2000	1	0.8	
2001	1	0.9	
2002	1	0.9	
2003	1	0.9	
2004	10	9.0	Epidemic with estimated 39 thousand rubella cases
2005	2	1.9	Maternal infection in India (1)
2006	0	0	2 dose-MR vaccination at 1 and 5 year-old
2007	0	0	
2008	0	0	Catch-up MR vaccination at 12 and 17 year-old (until 2012)
2009	2	1.9	Maternal infection in Philippine (1)
2010	0	0	
2011	1	1.0	Maternal infection in Vietnam (1)
2012	5	4.8	
2013	6		
Total	30		

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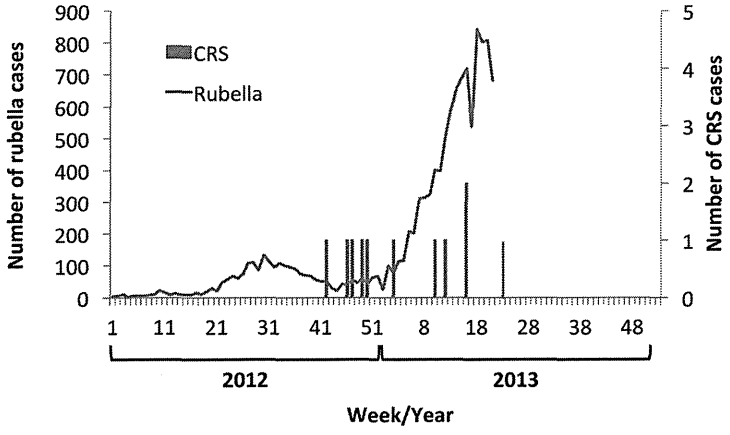
Weekly rubella and CRS cases (2012.1-2013.22)



IDWR 感染症発生動向調査週報

Summary

- 11 infants with CRS were reported between Jan. 2012 and Jun 2013 in Japan.
- The majority of rubella patients in the present epidemic are 20-40's men and 20's women, leading to concern about increase of CRS cases.

Nationalwide Rubella Epidemic – Japan, 2013. MMWR 62(23), 2013

Improving survival of Vero/hSLAM in the deep-freezing

Plans for a novel cell line for isolation of MV and RV

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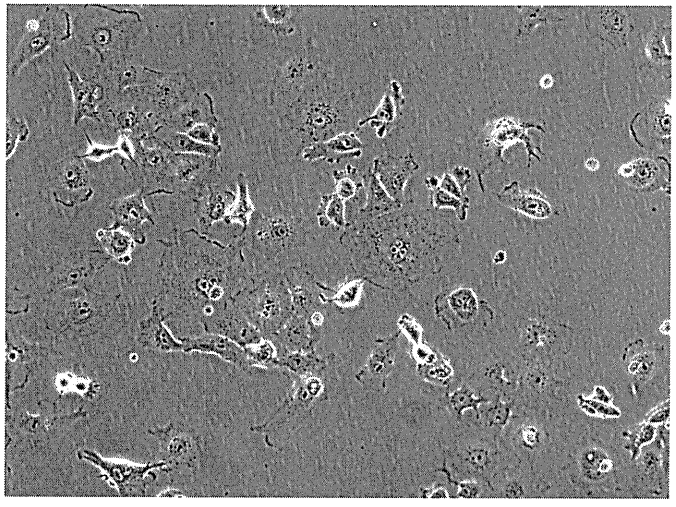
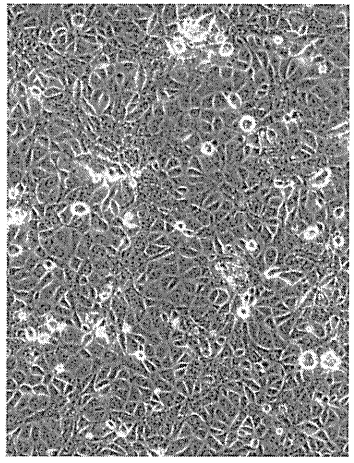
国立感染症研究所

Vero/hSLAM cells at 24 hours after thawing



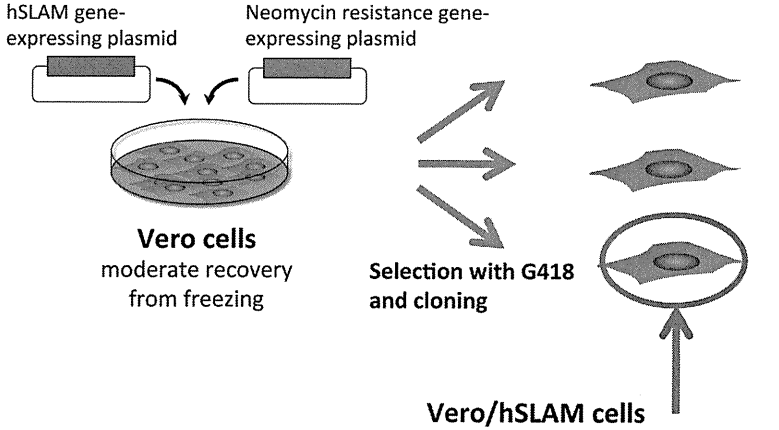
1×10^6 cells at ϕ60mm dish

Vero/hSLAM cells



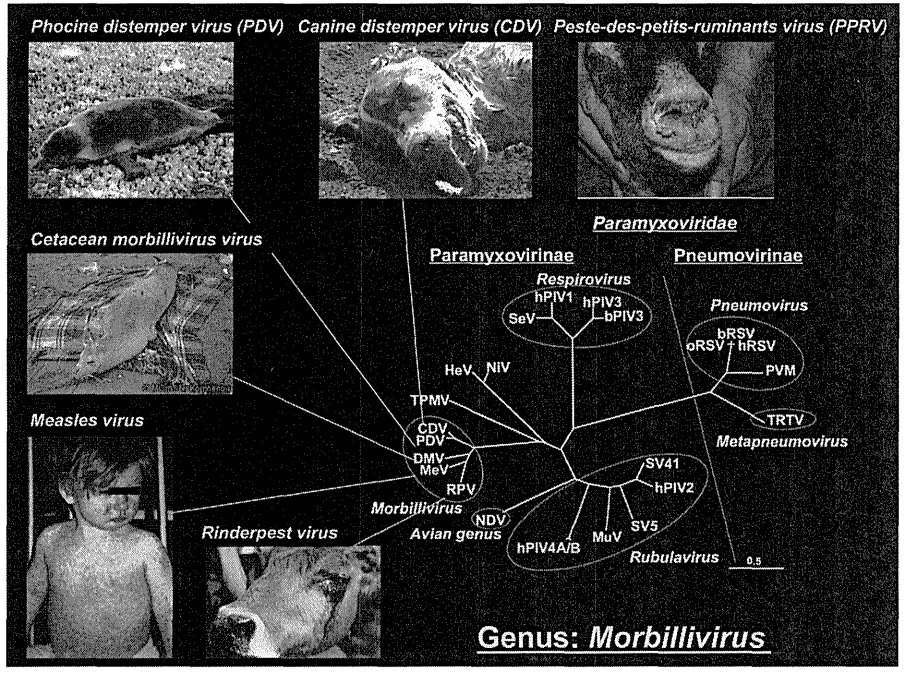
- Established by Dr. Yanagi's group (Ono et al, J. Virol., 2001)
- Stably express human SLAM, which is one of cellular receptors for wild type MV
- Useful for isolation of wild type MV
- Also available for isolation of RV
- Distributed to all over the world
- Low recovery from freeze stocking
- Regulated by CITES

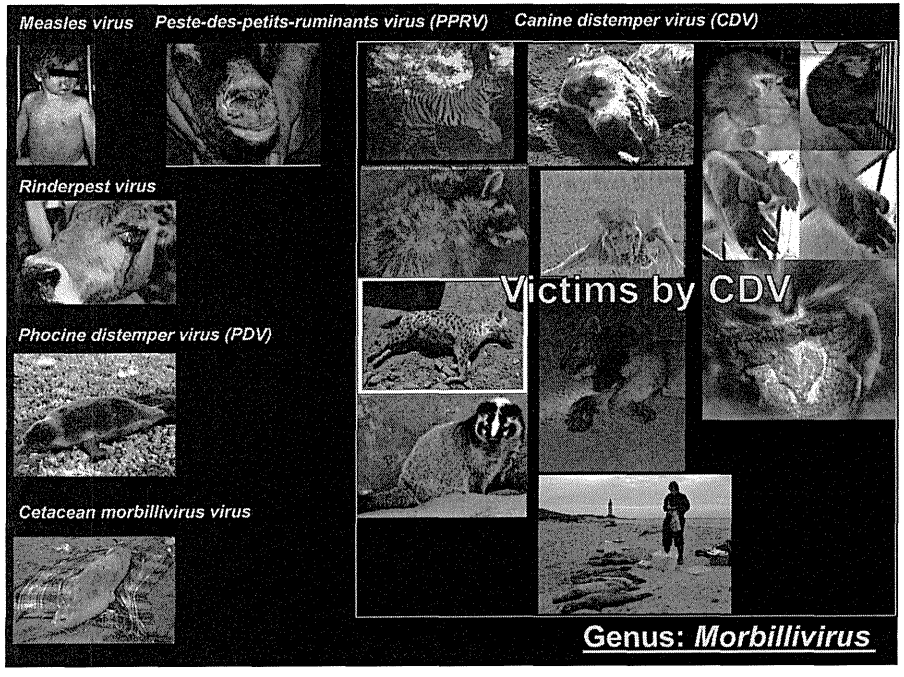
Method for establishment of Vero/hSLAM cells



To improve survival of Vero/hSLAM cells from deep-freezing

- Optimize methods for freezing and thawing (eg, cell density during freezing and plating just after thawing)
- Re-establishment of a new cell line based on high expression of hSLAM and high recovery rate from deep-freezing





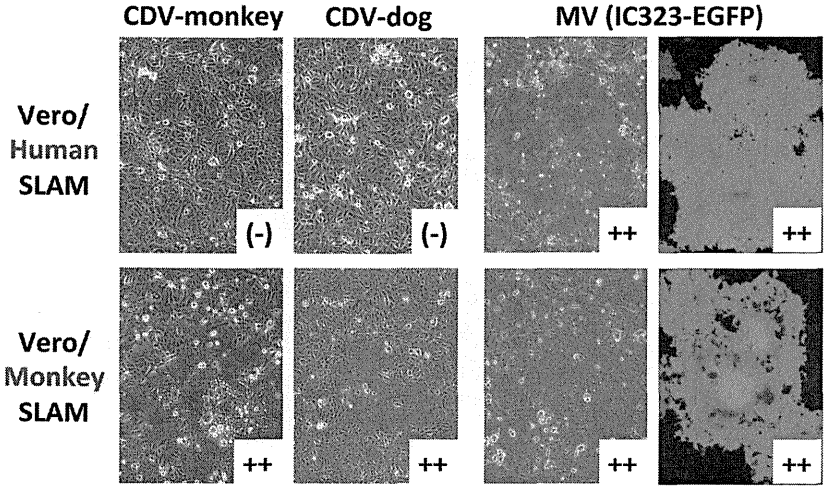
Recent CDV outbreaks in monkeys

Year	Location	Affected	Died	Mortality rate	Reference
2006-2008	China Guangxi	~ 10,000		5 - 30%	Que et al.
2008	China Beijing	20	12	60%	Sun et al.
2008	Japan	439	46	~ 10%	Sakai et al.

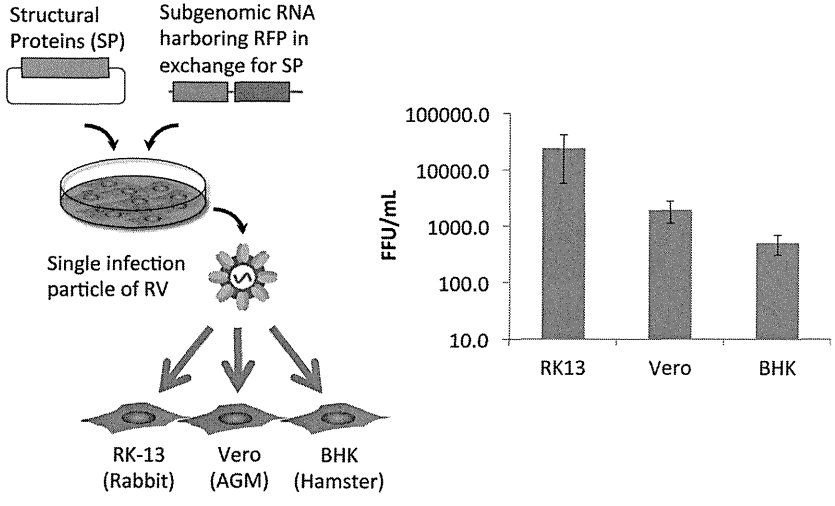
Qiu W et al. (2011) *Emerg Infect Dis* 17:1541-3.
Sun Z et al. (2010) *Vet Microbiol 141*:374-8.
Sakai K et al. (2013) *J Virol* 87:1105-14.

Qiu W et al. (2011)
Emerg Infect Dis 17:1541-3.

Monkey (Macaca) SLAM works as a receptor for MV and CDV



RK-13 rabbit cell line is highly susceptible to RV



Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

African Green Monkey
(*Cholorocebus aethiops*)

- Vero cells were originally established in 1962 from Kidney cells of African green monkey, which was a species listed on Appendix II of CITES.
- Formal procedures are required for international transportation of Vero cells.

Summary

- Vero/hSLAM cell line holds some problems.

  Low survival from deep-freezing
  CITES

- Establishment of a novel cell line for isolation of MV and RV are planned.

  Human SLAM or monkey SLAM?
  Vero or RK-13 cells?

11th WHO Global Measles and Rubella Laboratory Network Meeting
(24-26 June 2013, WHO HQ, Geneva)

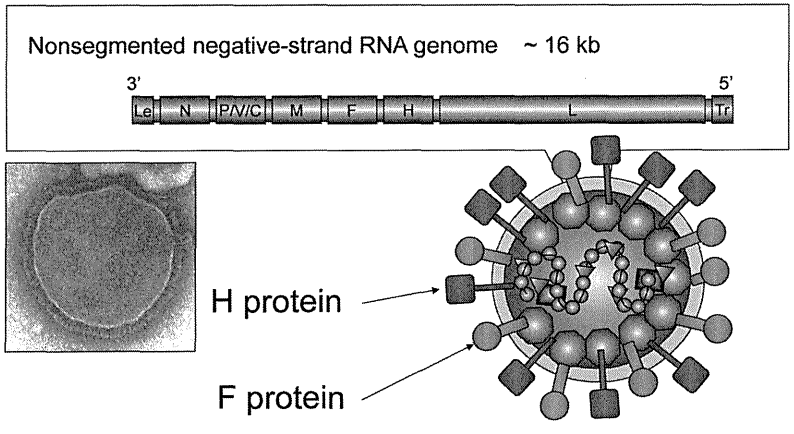
Functional Constraints on the Measles H Protein Prevent Escape from Neutralization

National Institute of Infectious Diseases
Dept. of Virology III
Katsuhiro Komase

2014年 1月 29日

24 genotypes of MV

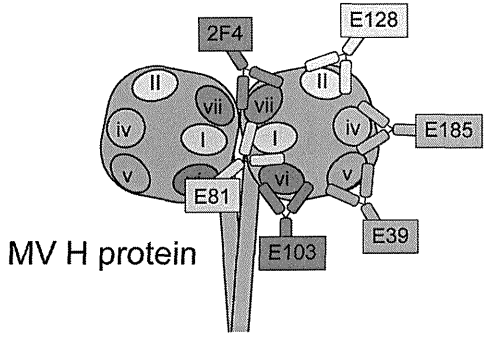
Measles virus



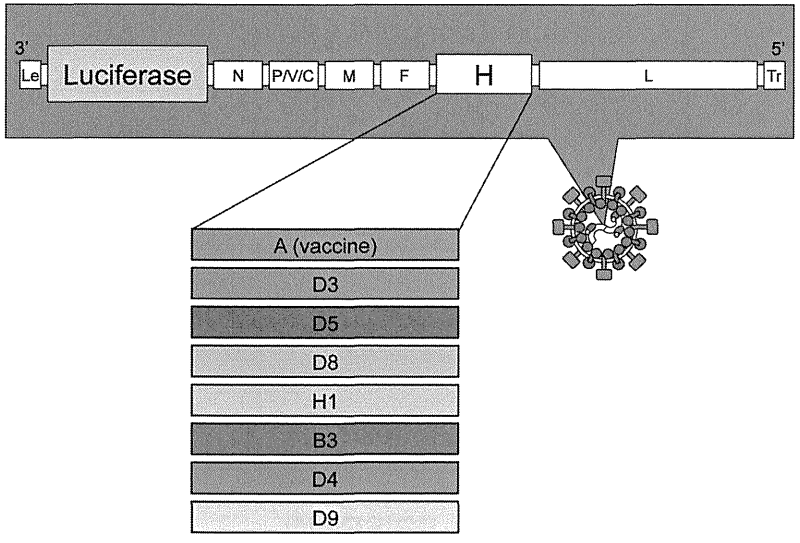
Antibody selection

mAbs used in the present study

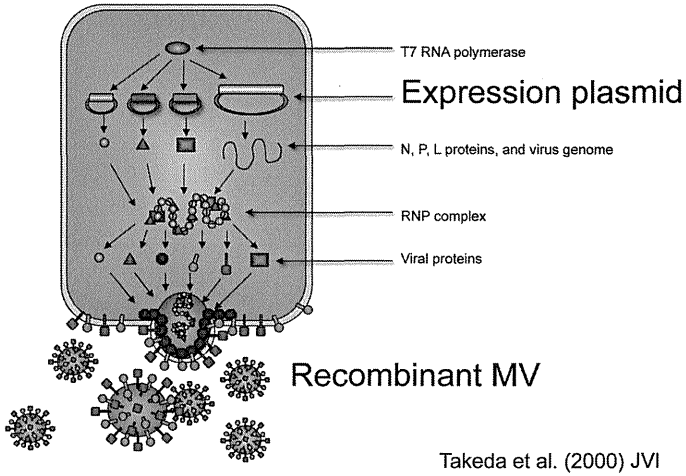
epitope	I	II	iv	v	vi	vii
mAb	E81	E128	E185	E39	E103	2F4



Generation of neutralizing targets (recombinant MVs)

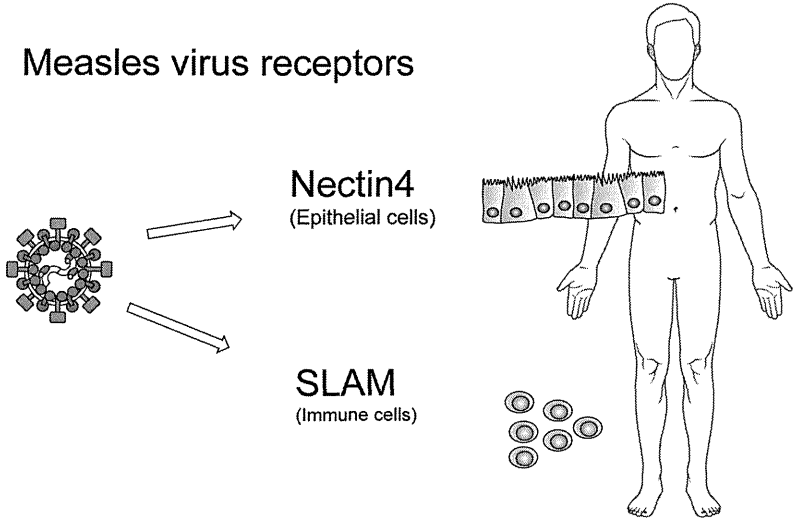


Reverse genetics system to engineer MV

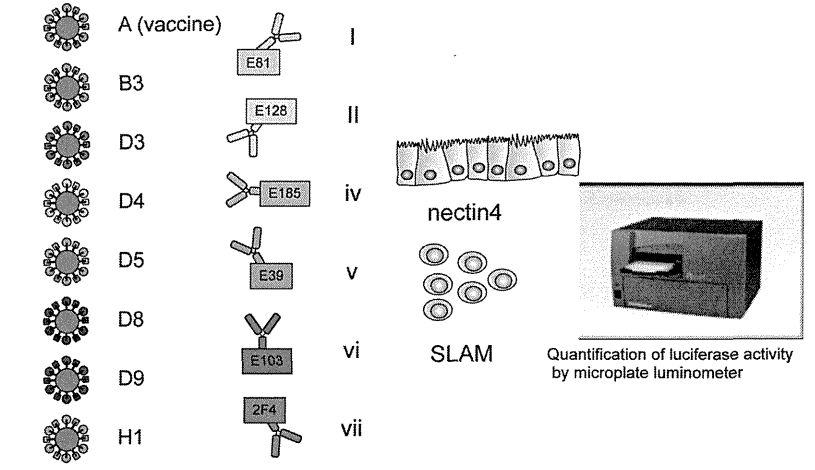


Takeda et al. (2000) JVI

Measles virus receptors



Luciferase-based quantitative neutralization assay



8 genotypes (H protein) × 6 epitopes (mAbs) × 2 cell lines (Receptors) = 96 combinations (neutralizations)

Neutralization titers of 96 combinations

SLAM-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
B95a	A (vaccine)	863	1968	494	<27	2558	22141
	B3	1727	1968	987	<27	10231	11070
	D3	1727	15	31	<27	10231	22141
	D4	1727	31	62	<27	20462	22141
	D5	863	15	494	<27	10231	11070
	D8	1727	1968	987	<27	20462	44281
	D9	1727	15	987	<27	20462	11070
	H1	1727	1968	494	<27	20462	22141

Nectin4-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
II-18	A (vaccine)	27631	62977	124	1750	10231	11070
	B3	27631	62977	1974	1750	10231	11070
	D3	27631	123	62	<27	20462	11070
	D4	27631	31	<8	<27	10231	11070
	D5	27631	<4	1974	<27	20462	22141
	D8	27631	62977	987	<27	10231	22141
	D9	27631	123	1974	<27	10231	11070
	H1	13815	62977	494	1750	10231	22141

Epitopes I, vi and vii are conserved effective neutralizing epitopes

SLAM-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
B95a	A (vaccine)	863	1968	494	<27	2558	22141
	B3	1727	1968	987	<27	10231	11070
	D3	1727	15	31	<27	10231	22141
	D4	1727	31	62	<27	20462	22141
	D5	863	15	494	<27	10231	11070
	D8	1727	1968	987	<27	20462	44281
	D9	1727	15	987	<27	20462	11070
	H1	1727	1968	494	<27	20462	22141

Nectin4-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
II-18	A (vaccine)	27631	62977	124	1750	10231	11070
	B3	27631	62977	1974	1750	10231	11070
	D3	27631	123	62	<27	20462	11070
	D4	27631	31	<8	<27	10231	11070
	D5	27631	<4	1974	<27	20462	22141
	D8	27631	62977	987	<27	10231	22141
	D9	27631	123	1974	<27	10231	11070
	H1	13815	62977	494	1750	10231	22141

Epitopes II is an effective neutralizing epitope, but not conserved.

SLAM-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
B95a	A (vaccine)	863	1968	494	<27	2558	22141
	B3	1727	1968	987	<27	10231	11070
	D3	1727	15	31	<27	10231	22141
	D4	1727	31	62	<27	20462	22141
	D5	863	15	494	<27	10231	11070
	D8	1727	1968	987	<27	20462	44281
	D9	1727	15	987	<27	20462	11070
	H1	1727	1968	494	<27	20462	22141

Nectin4-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
II-18	A (vaccine)	27631	62977	124	1750	10231	11070
	B3	27631	62977	1974	1750	10231	11070
	D3	27631	123	62	<27	20462	11070
	D4	27631	31	<8	<27	10231	11070
	D5	27631	<4	1974	<27	20462	22141
	D8	27631	62977	987	<27	10231	22141
	D9	27631	123	1974	<27	10231	11070
	H1	13815	62977	494	1750	10231	22141

Epitopes iv and v are less important epitopes.

SLAM-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
B95a	A (vaccine)	863	1968	494	<27	2558	22141
	B3	1727	1968	987	<27	10231	11070
	D3	1727	15	31	<27	10231	22141
	D4	1727	31	62	<27	20462	22141
	D5	863	15	494	<27	10231	11070
	D8	1727	1968	987	<27	20462	44281
	D9	1727	15	987	<27	20462	11070
	H1	1727	1968	494	<27	20462	22141

Nectin4-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
II-18	A (vaccine)	27631	62977	124	1750	10231	11070
	B3	27631	62977	1974	1750	10231	11070
	D3	27631	123	62	<27	20462	11070
	D4	27631	31	<8	<27	10231	11070
	D5	27631	<4	1974	<27	20462	22141
	D8	27631	62977	987	<27	10231	22141
	D9	27631	123	1974	<27	10231	11070
	H1	13815	62977	494	1750	10231	22141

Epitopes I, vi and vii are conserved among these 8 genotypes

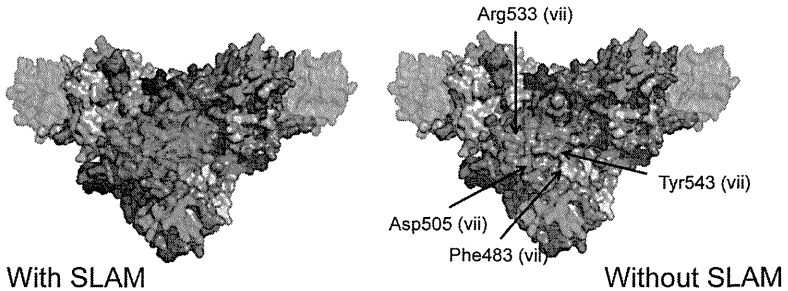
SLAM-mediated infection

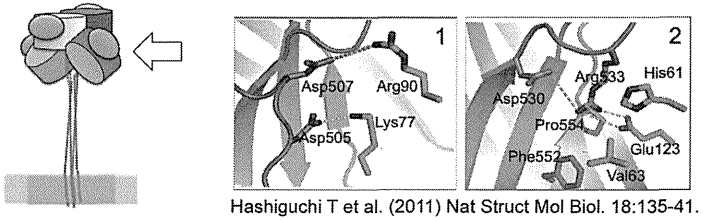
	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
B95a	A (vaccine)	863	1968	494	<27	2558	22141
	B3	1727	1968	987	<27	10231	11070
	D3	1727	15	31	<27	10231	22141
	D4	1727	31	62	<27	20462	22141
	D5	863	15	494	<27	10231	11070
	D8	1727	1968	987	<27	20462	44281
	D9	1727	15	987	<27	20462	11070
	H1	1727	1968	494	<27	20462	22141

Nectin4-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
II-18	A (vaccine)	27631	62977	124	1750	10231	11070
	B3	27631	62977	1974	1750	10231	11070
	D3	27631	123	62	<27	20462	11070
	D4	27631	31	<8	<27	10231	11070
	D5	27631	<4	1974	<27	20462	22141
	D8	27631	62977	987	<27	10231	22141
	D9	27631	123	1974	<27	10231	11070
	H1	13815	62977	494	1750	10231	22141

Epitope vii (conserved epitope)

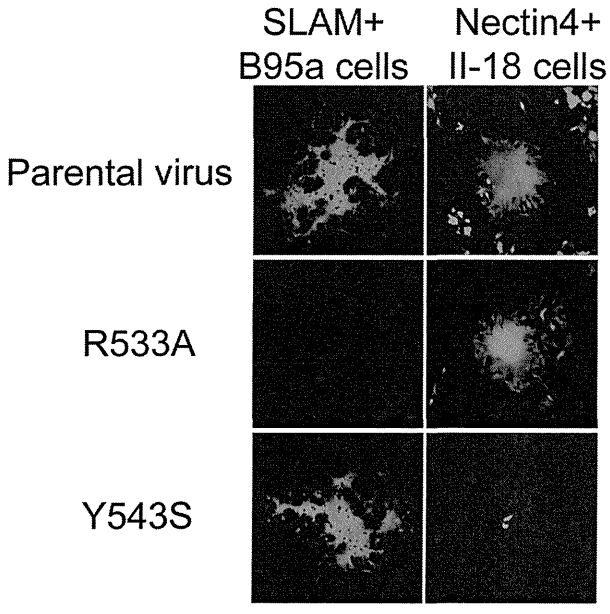




Hashiguchi T et al. (2011) Nat Struct Mol Biol. 18:135-41.

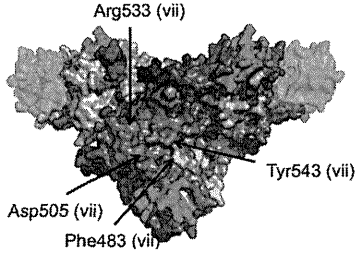
Linkage between the growth defect and escape

	Mutation	Neutralization titer			Growth in	
		I	vi	vii	B95a	II-18
Cell		E81	E103	2F4	SLAM	Nectin4
B95a		1727	10231	22141	++	++
	F483A	1727	10231	<346	++	(-)
	Y543S	1727	2558	692	++	(-)
II-18		27631	20462	11070	++	++
	D505S	13815	20462	<5	(-)	++
	R533A	27631	20462	173	(-)	++



Summary of the conserved epitope vii

- Receptor-interacting residues themselves constitute an important neutralizing epitope.
- Consequently, MV became incapable of utilizing either SLAM or nectin4, when it possessed an escape mutation on the epitope.



Epitopes I, vi and vii are conserved effective neutralizing epitopes

SLAM-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
B95a	A (vaccine)	863	1968	494	<27	2558	22141
	B3	1727	1968	987	<27	10231	11070
	D3	1727	15	31	<27	10231	22141
	D4	1727	31	62	<27	20462	22141
	D5	863	15	494	<27	10231	11070
	D8	1727	1968	987	<27	20462	44281
	D9	1727	15	987	<27	20462	11070
	H1	1727	1968	494	<27	20462	22141

Nectin4-mediated infection

	epitope	I	II	iv	v	vi	vii
	IgG 1 mg/ml	E81	E128	E185	E39	E103	2F4
II-18	A (vaccine)	27631	62977	124	1750	10231	11070
	B3	27631	62977	1974	1750	10231	11070
	D3	27631	123	62	<27	20462	11070
	D4	27631	31	<8	<27	10231	11070
	D5	27631	<4	1974	<27	20462	22141
	D8	27631	62977	987	<27	10231	22141
	D9	27631	123	1974	<27	10231	11070
	H1	13815	62977	494	1750	10231	22141

Comparison of neutralizing titers by mAbs and vaccinees' serum

II-18 cell	mAbs*					
	E81 (I)	E128 (II)	E185 (iv)	E39 (v)	E103 (vi)	2F4 (vii)
A	27631	62977	124	1750	10231	11070
D3	27631	123	62	<27	10231	11070
D3 (Q311R/ Q391R)	<3	ND**	ND	ND	20	11070
D3 (Q311R/ Q391R/R533A)	3	ND	ND	ND	40	346
D3 (Q311R/ Q391R/D505S)	<3	ND	ND	ND	20	<5

*1mg/ml of Ig. **Very low (titers were not determined)

Antibody: >25000 | 10000-25000 | 5000-10000 | 2500-5000 | 1000-2500 | <1000

Comparison of neutralizing titers by mAbs and vaccinees' serum

II-18 cell	mAbs*						Vaccinees						
	E81 (I)	E128 (II)	E185 (iv)	E39 (v)	E103 (vi)	2F4 (vii)	Vac9	Vac12	Vac27	Vac31	Vac40	Vac41	Vac49
A	27631	62977	124	1750	10231	11070	80	640	80	80	80	160	160
D3	27631	123	62	<27	10231	11070	160	640	80	160	160	160	320
D3 (Q311R/ Q391R)	<3	ND**	ND	ND	20	11070							
D3 (Q311R/ Q391R/R533A)	3	ND	ND	ND	40	346							
D3 (Q311R/ Q391R/D505S)	<3	ND	ND	ND	20	<5							

*1mg/ml of Ig. **Very low (titers were not determined)

Antibody: >25000 | 10000-25000 | 5000-10000 | 2500-5000 | 1000-2500 | <1000

Serum: 640 | 320 | 160 | 80 | 40 | <40

Comparison of neutralizing titers by mAbs and vaccinees' serum

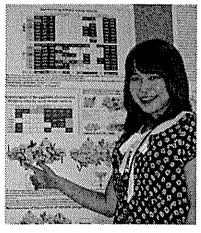
II-18 cell	mAbs*						Vaccinees						
	E81 (*I*)	E128 (*II*)	E185 (*iv*)	E39 (*v*)	E103 (*vi*)	2F4 (*vii*)	Vac9	Vac12	Vac27	Vac31	Vac40	Vac41	Vac49
A	27631	62977	124	1750	10231	11070	80	640	80	80	80	160	160
D3	27631	123	62	<27	10231	11070	160	640	80	160	160	160	320
D3 (Q311R/ Q391R)	<3	ND**	ND	ND	20	11070	80	320	160	160	320	320	320
D3 (Q311R/ Q391R/R533A)	3	ND	ND	ND	40	346	160	320	160	160	160	320	320
D3 (Q311R/ Q391R/D505S)	<3	ND	ND	ND	20	<5	160	640	80	320	320	320	320

*1mg/ml of Ig. **Very low (titers were not determined)

Antibody	>25000	10000-25000	5000-10000	2500-5000	1000-2500	<1000
Serum	640	320	160	80	40	<40

- MVs are neutralized by vaccinees' serum, even at the cost of reduced affinity for one of the receptors or lower replication capacity.
- MV does not undergo a major antigenic drift, and global measles elimination is biologically feasible with currently used MV vaccines.

Acknowledgements



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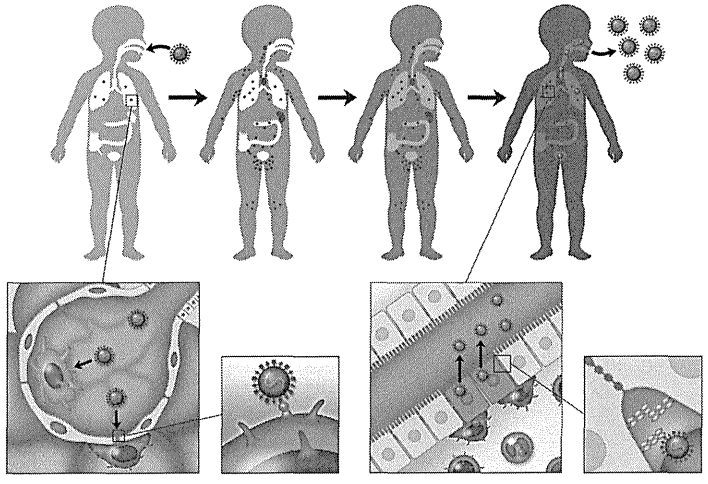

Escape mutations from 2F4

		Mutation	Neutralization titer		
	epitope		I	vi	vii
Cell			E81	E103	2F4
B95a	D3/		1727	10231	22141
	D3/	F483A	1727	10231	<346
	D3/	Y543S	1727	2558	692
Il-18	D3/		27631	20462	11070
	D3/	D505S	13815	20462	<5
	D3/	R533A	27631	20462	173