

Dried Blood and Filter Paper Alternative Sampling Techniques for Measles Cases Confirmation (CIV)

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Introduction

- └ **Measles : child hood morbidity / mortality (Africa, Asia)**
- └ **Diagnosis based on clinical symptoms**
 - └ **Typical / atypical**
- **Laboratory diagnosis plays critical role for Measles elimination / control programme**
- **Specific IgM in serum = gold standard**
- **Collection of blood has significant limitations**
- **New approaches- Dried blood spot and oral fluid tools for IgM and viral genome detection are gaining popularity.**



Measles profile (DC-PEV)

Years	Coverage	Reported cases	Reported death
2001	62%	5790	NR
2002	60%	7633	NR
2003	47%	5207	105

NR : No reported



Aims

- └ **Set up use of ELISA kits for testing dried blood and oral fluid**
- **Evaluate sensitivity and specificity for detecting measles IgM in dried blood / oral fluid**
- **Evaluate operational issues around the use of dried spots and oral fluid**



Patients

- └ Measles suspected cases (WHO case definition)
→ EPI surveillance system
- └ Laboratory research team trained on measles investigation procedures contacts the staff of the district
- District staff informs populations in the area and takes investigators to the place
- Each measles suspected patient is seen and examined, & informed consent obtained from the parent.



Specimens collection

- ┌ **Within 28 days after rash onset date, from each suspected patient :**
 - ┌ **Blood sample for serum**
 - **Dried blood spot**
 - **Oral fluid**
- **Patient is held firmly by the parent**



- └ **Serum : serum venous blood collection**
 - └ Venepuncture in sterile labelled tube
 - └ Shipment to laboratory
 - └ Centrifugation
 - └ Serum frozen at – 20°C
- **Oral fluid collection**
 - With a special swab (OraCol) using as a toothbrush to collect crevicular fluid from the mounth
 - The wet swab is placed inside a clear plastic tube labelled, shipped within 48 hours to the lab.
 - The oral fluid is extracting from the swab using a special transport medium and stored at – 20°C
- **Dried blood collection :**
 - Skin puncture on the finger or heel
 - One drop is put on each circle of a filter paper labelled
 - Allow the filter paper to dry thoroughly before enclosing in a plastic bag or envelope.
 - Storage at – 20°C

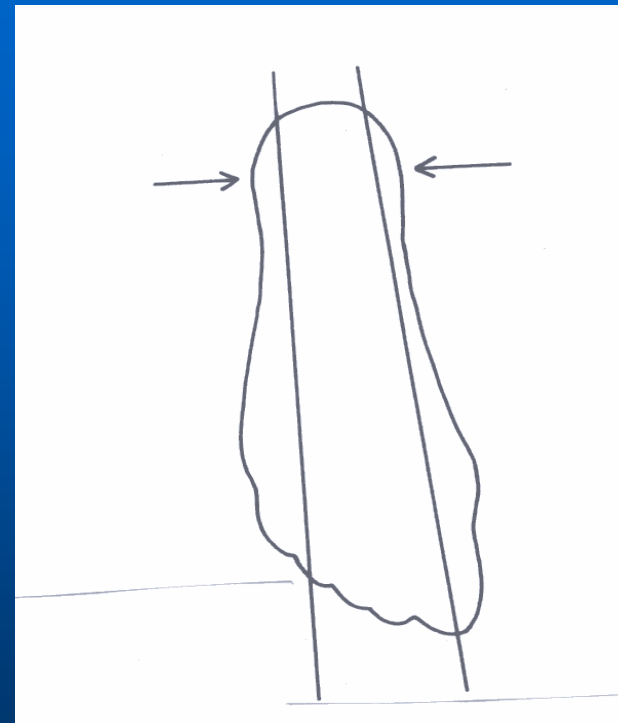


Diagram of Filter paper collection card

Laboratory No.



Dried blood collection : Recommended sites for finger and heel



1-Filter paper sample collection method: peripheral blood- IgM and PCR

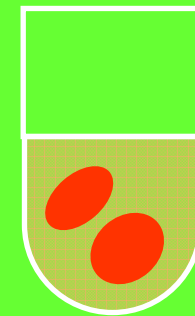
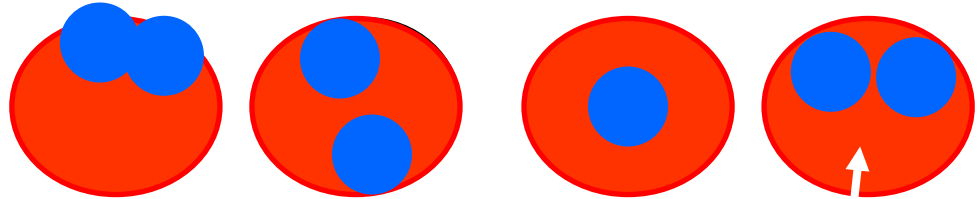
Name:.....
DOB:.....
Date collection:.....

Name: Mizelle Elim

Age: 12 mths M(F) Date collection: 12/2/03

Laboratory Number

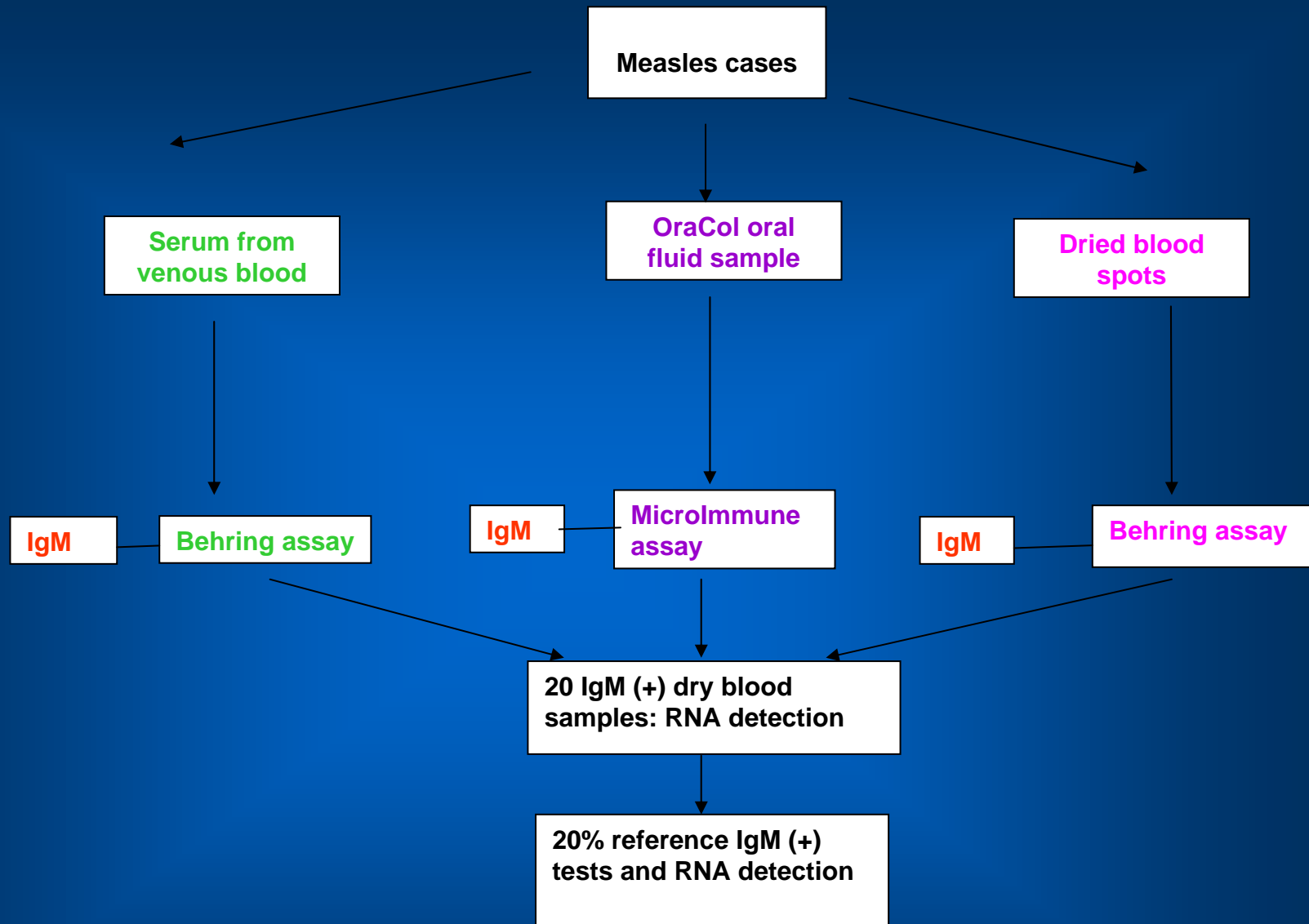
03/2437



Not this way!

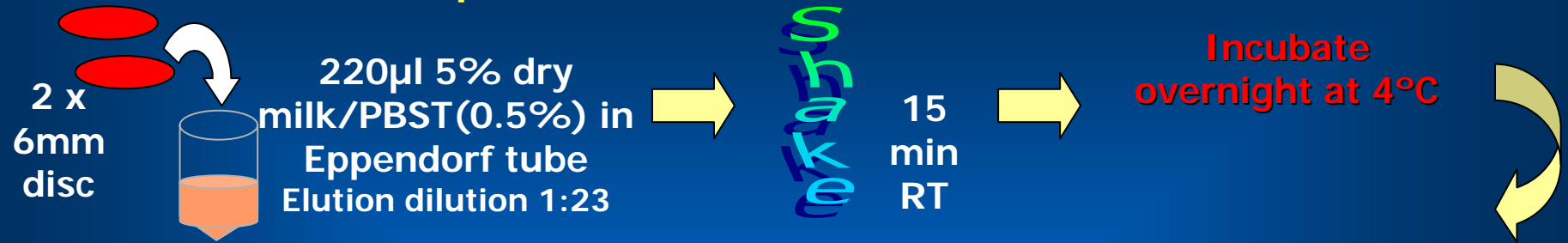
This way



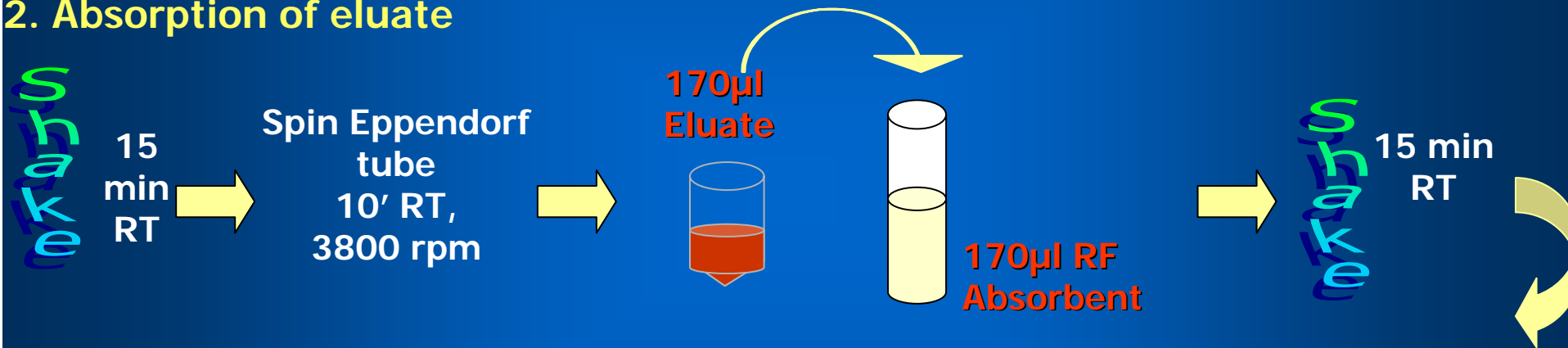


Elution of dried blood spots for Behring Measles IgM assay

1. Elution of dried blood spot



2. Absorption of eluate



3. Immunoassay – Dade Behring Enzygnost® Anti-Measles-Virus/IgM



Dried blood Immunoassay

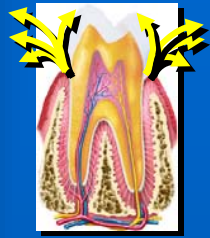
└ Washing

└ Protocole recommendation : wash X 3

└ Wash X5 instead of 3



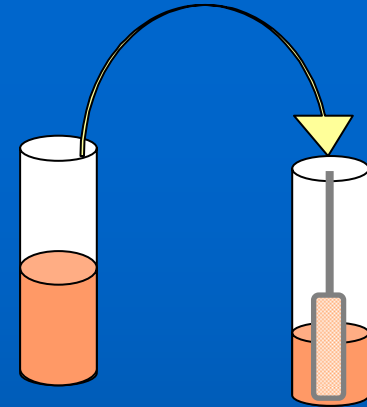
Collection and processing of oral fluid sample



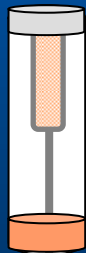
Use Orocol swab like a tooth brush; 1 minute



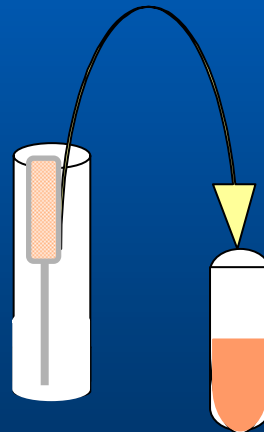
Seal in tube and send to lab



Add 1 ml extraction buffer and mix well



Use forceps to reverse swab and centrifuge 2,000 rpm 5 mins



Discard swab and transfer supernatant to cryovial



Test for IgM, undiluted, in MicroImmune assay

Results

- └ From May 2002 to July 2004
- └ 182 measles suspected cases
 - └ 2002 : 27 (14,8%)
 - 2003 : 73 (40,1%)
 - 2004 : 82 (45,1%)
- 16 districts
- Samples
 - 176 sera
 - 176 dried blood spots
 - 135 oral fluid



Dried Blood Results

CIV Study:

Summary of Measles-IgM Results in Dried Blood Filter Paper / Serum Sample (Dade Behring^R, N=176)

Serum

	IgM+	IgM-	Indeterm.
Dried blood IgM+	97	5	8
IgM-	0	53	2
Indeterm.	4	2	5

True Concordance: = 155/176 = 88.1%

Rel. Sensitivity = 97/97 = 100%

Rel. Specificity = 53/58 = 91.4%

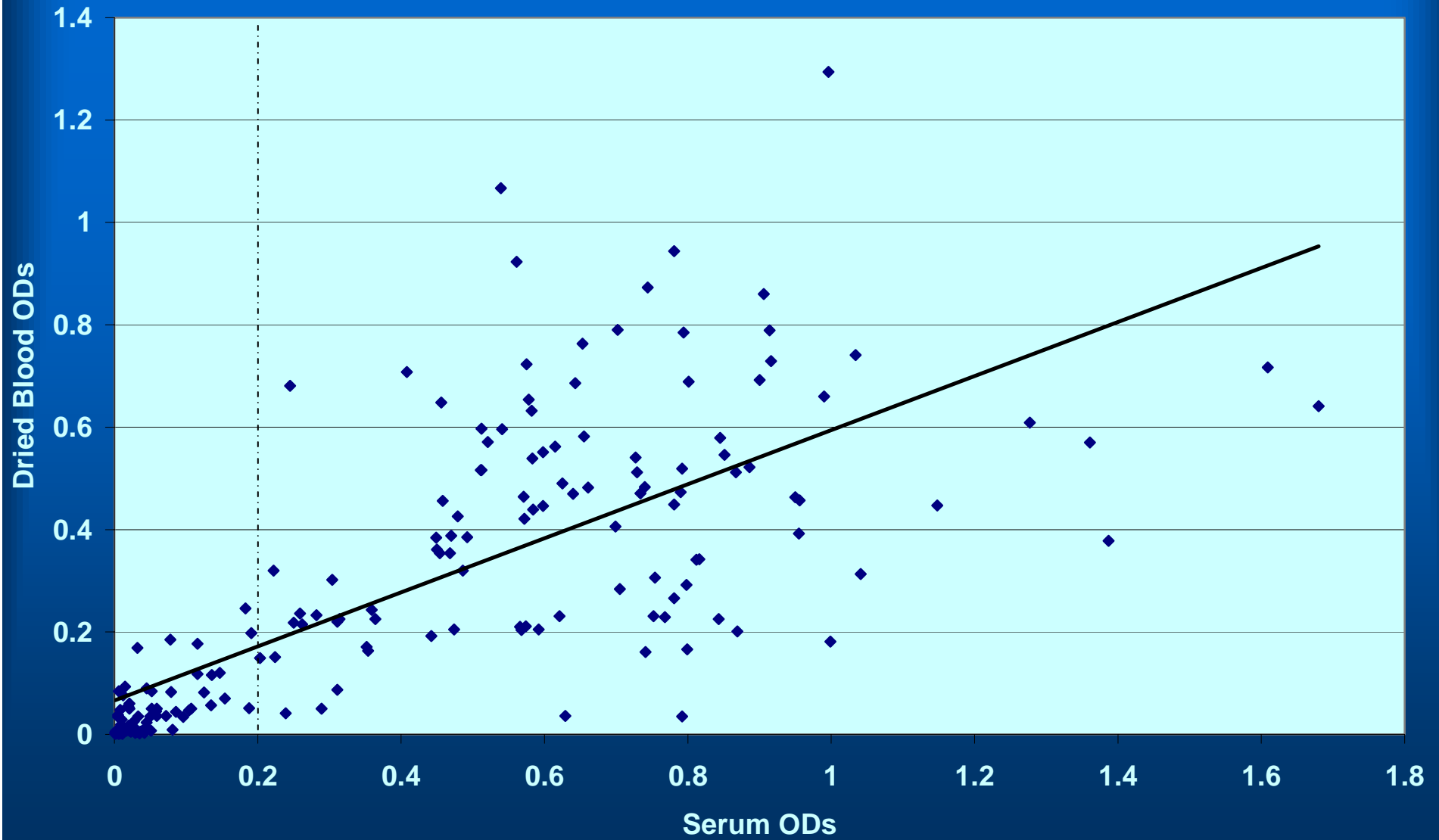
Pos. Pred. Value = 97/102 = 95.1%

Neg. Pred. Value = 53/53 = 100%

Indeterminates ignored

RESULTS July 04

CIV Dried Blood vs. Serum N=175



RESULTS July 04

Oral Fluid Results

CIV: Measles-IgM Results in oral fluid / serum sample pairs (Microimmune^R /Behring^R, N= 135)

Serum

IgM+ IgM- Indeterm.

Oral fluid

IgM+

74	4	3
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IgM-

3	45	6
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Indeterm.

0	0	0
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True Concordance: = 119/135 = 88.1%

Rel. Sensitivity = 74/77 = 96.1%

Rel. Specificity = 45/49 = 91.8%

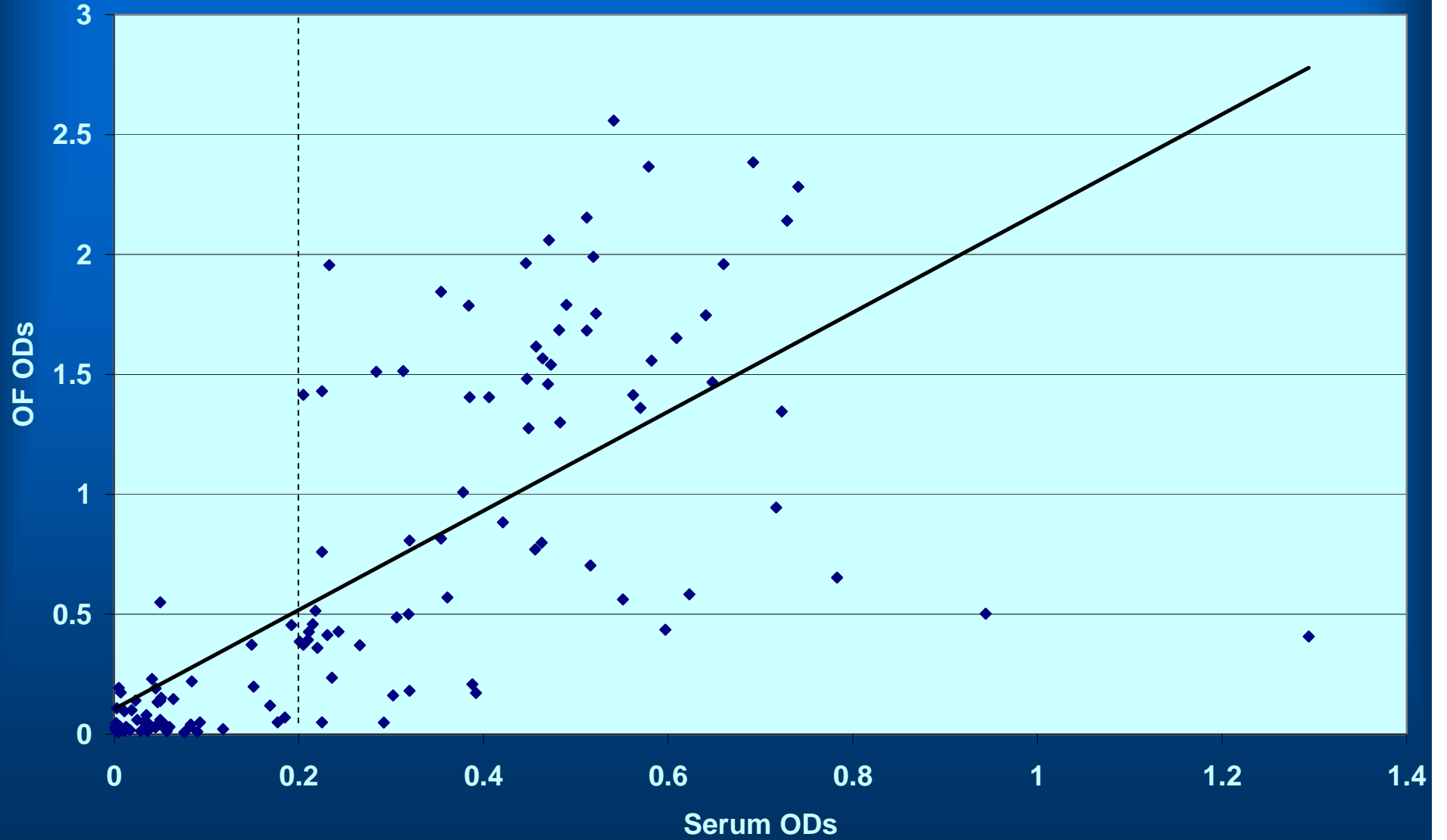
Pos. Pred. Value = 74/78 = 94.9%

Neg. Pred. Value = 45/48 = 93.8%

Indeterminates ignored

RESULTS Jul 04

CIV Oral fluid vs. Serum N=130



RESULTS JUL 04

Observations

└ Sensitivity :

- └ Dried blood : 100%
- └ Oral fluid : 96,1%

■ Specificity : Dried Blood \approx Oral fluid

- Se : 91,4% / 91,8%

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■ Precautions are needed :

- Prompt delivery
- Training for collection, drying + storage
- Eluation



Operational issues

Specimen	Collection	Testing
Dried blood spots	Prons : Convenient Cons: Filling full disc diameter. Must dry well	* Appropriate centrifuge facilities * Wash X5 instead of 3
Oral fluid	No Transport Medium : prompt delivery essential	Simple



**Detection of measles virus in
dried blood and oral fluid by
RT-PCR**

CIV: Nested RT-PCR of Dried Blood samples (subset N=15)

Lab ref.	Date of onset of rash	Date of collection	Days post rash	PCR measles positive (nested)
R038/03	8 / 02 / 03	14 / 02 / 03	6	+
R148/03	2 / 4 / 03	7 / 4 / 03	5	+
R161/03	4 / 4 / 03	9 / 4 / 03	5	+
R162/03	4 / 4 / 03	9 / 4 / 03	5	+
R165/03	4 / 4 / 03	9 / 4 / 03	5	-
R172/03	9 / 4 / 03	9 / 4 / 03	0	+
R197/03	3 / 4 / 03	10 / 4 / 03	7	+
R214/03	7 / 4 / 03	11 / 4 / 03	4	+
R217/03	5 / 4 / 03	11 / 4 / 03	6	+
R218/03	5 / 4 / 03	11 / 4 / 03	6	+
R227/03	9 / 4 / 03	11 / 4 / 03	2	+
R303/03	12 / 4 / 03	19 / 4 / 03	7	+
R304/03	12 / 4 / 03	19 / 4 / 03	7	+
R320/03	28 / 4 / 03	2 / 5 / 03	4	+
R323/03	25 / 4 / 03	2 / 5 / 03	7	+

Nested RT-PCR

- ┌ **Sensitivity : 93,3%**
- ┌ **Specificity : 100%**



Conclusion

- └ < 176 cases tested
- └ ≥ 50% confirmed measles cases
- Dried blood / oral fluid
 - Good sensitivity
 - Specificity : 91%
- RNA detection : good specificity
- Dried blood spots/oral fluid are interesting tools but some precautions and facilities are needed.

