

Laboratory Challenges in Infant Diagnosis:

Dried Blood Spot for Early Infant
Diagnosis Using DNA PCR in Rwanda

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What Is a Dried Blood Spot (DBS)?

- Whole blood dried on filter paper
- Usually obtained from pricking skin, not from phlebotomy
- Requires only a small amount of blood
- Easy to store
- Easy to transport



Required Supplies for DBS

- Blood collection card (filter paper)
- Lancet
- Alcohol
- Gauze or cotton wool
- Sealable plastic bags
- Humidity cards
- Desiccant packs

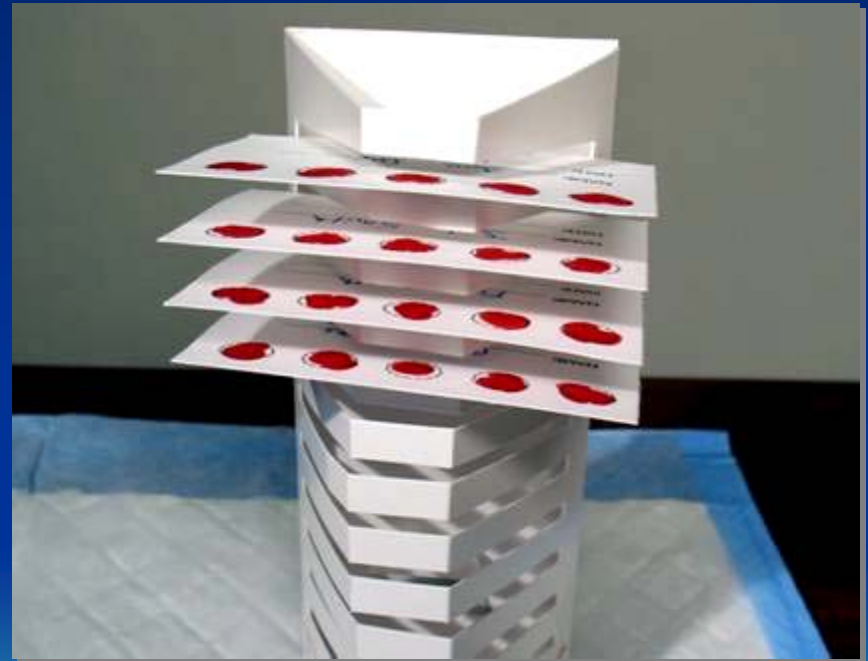
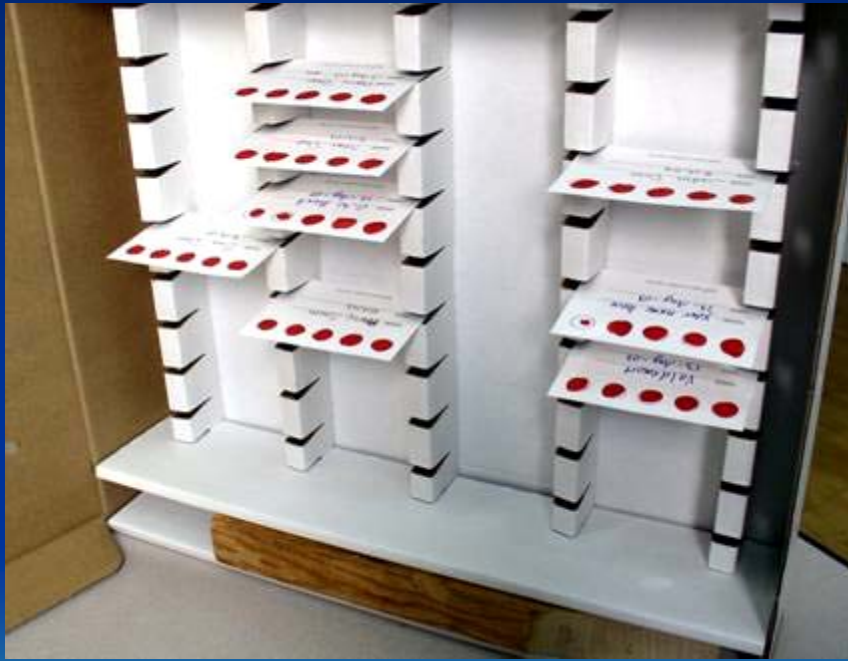


How to Collect DBS

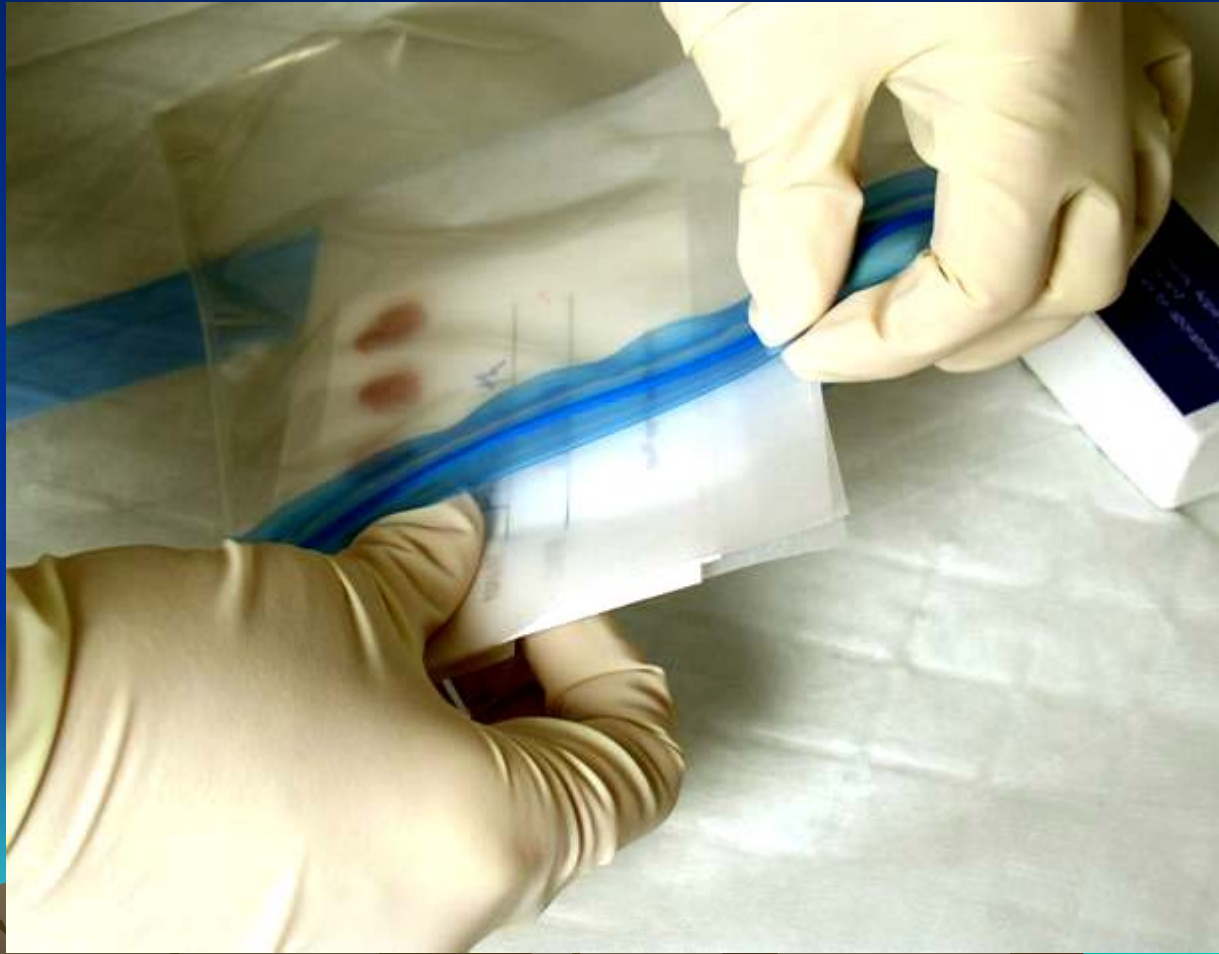
- Use Universal Safety Precautions for handling blood
- Clearly label card with appropriate identification number
- Do not touch circles



Dry Completely Before Packaging



Insert Into Sealable Plastic Bag



Add Desiccant Packets

Minimum 10 packets per bag



Add Humidity Cards and Seal Bag



How to Package DBS for Shipping

1. Insert into envelope
2. Include lab requisitions
3. Include specimen delivery checklist
4. Label outside clearly
5. Send to NRL



Early infant diagnosis: why is it important?

For the health of infants

- High mortality among HIV+ infants

Rapid progressor = infected in uterus and Peri-natal

- First significant illness likely to end in death
- Infants with HIV do not grow well, and may suffer brain damage
- *Early treatment improves outcomes – treat before they are sick*



Early infant diagnosis: why is it important?

For assessment of the PMTCT program

- 18 month testing cannot accurately show program effectiveness
 - Many positive babies have died
 - Most babies cannot be found
 - Poor records of what PMTCT interventions were given
- Early testing gives more accurate program effectiveness data



Diagnosing HIV in infants

- ELISA tests
 - Inexpensive
 - Accurate only after 18 months
 - Require phlebotomy
- Rapid tests
 - Inexpensive
 - Accurate only after 18 months
- PCR (DNA or RNA)
 - More expensive
 - Accurate even at birth
 - In the past, required phlebotomy
 - Now can be done on blood from a heel prick

Diagnosing HIV in infants

- P24 Ultra sensible
 - Performance in subtypes Non B need to be assessed
 - Not yet validate in DBS.
- Viral Load
 - Not all have good performance in subtype Non-B
 - More expensive



Early infant diagnosis: technical background

- Until now, only ELISA and rapid tests have been widely available
- ELISA and rapid testing not accurate in a baby under 18 months
 - Tests do not look for HIV virus
 - They look for HIV antibody, made by a person to fight the HIV virus
 - All babies from HIV+ mothers have mother's HIV antibody at birth
 - Mother's antibody goes away if baby is not infected

Early infant diagnosis: technical background

- ELISA and rapid tests
 - By 18 months antibody is gone in HIV negative babies
 - At this point ELISA or rapid tests are quite accurate



Early infant diagnosis: technical background

- PCR testing
 - Stands for Polymerase Chain Reaction
 - Looks for HIV virus DNA, not antibody
 - Not a new test
 - Used in US for 15 years
 - Used in Rwanda since 2003, but usually by pediatricians – required phlebotomy
 - Quite accurate even in newborns
 - Almost 100% accurate after 6 weeks old



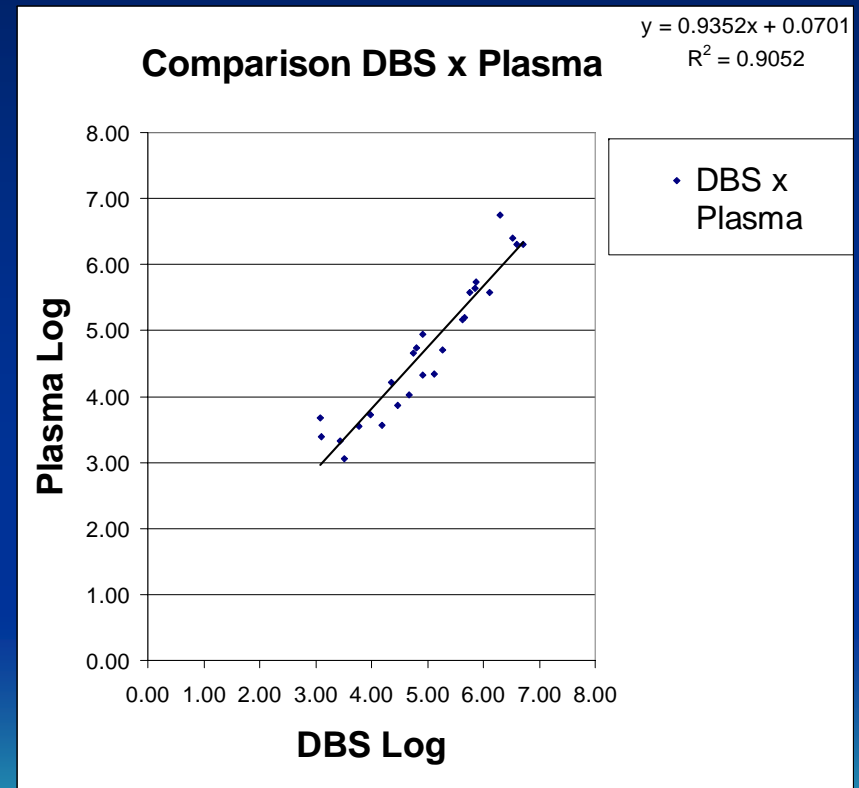
Background work in Rwanda Pilot

- We developed a highly efficient method for HIV proviral DNA extraction from DBS samples using the extraction procedure from Amplicor v 1.5 kit for Roche
- We also proved that DNA is very stable in DBS.
- We also proved in field evaluation conducted in Rwanda in Feb 05 that the DNA PCR can be used with success in patient's samples.



1st phase DBS Evaluation

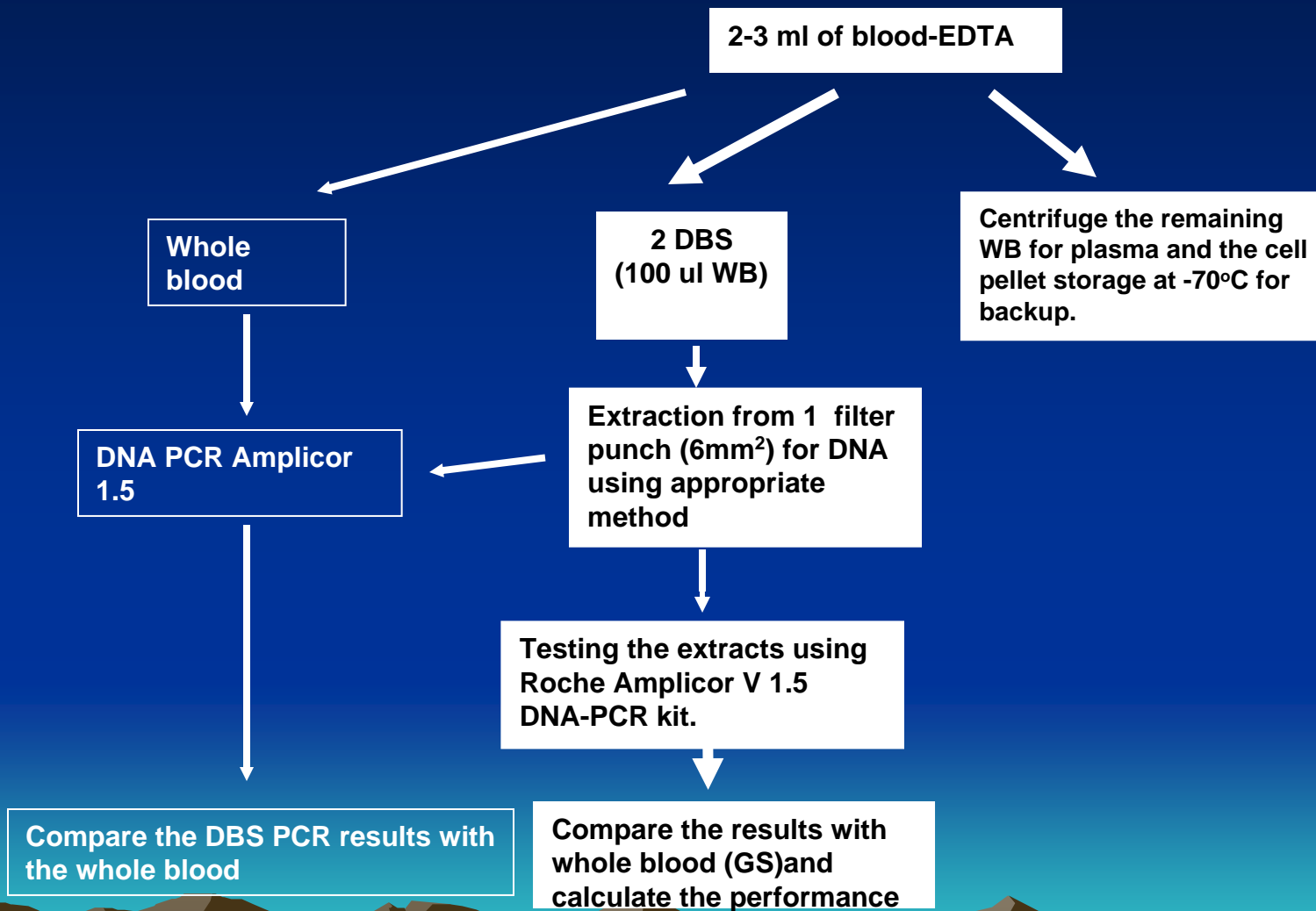
DBS	PLASMA	DNA PCR Whole Blood	DNA PCR DBS	SAMPLE
LLD	LLD	neg	neg	5
LLD	LLD	neg	neg	9
LLD	LLD	neg	neg	10
LLD	LLD	neg	neg	16
LLD	LLD	neg	neg	21
LLD	LLD	neg	neg	23
LLD	LLD	neg	neg	25
LLD	LLD	neg	neg	27
LLD	LLD	neg	neg	35
LLD	LLD	neg	neg	37
LLD	LLD	neg	neg	38
3.06	3.51	neg	neg	26
3.33	3.43	neg	neg	34
3.39	3.10	neg	neg	28
3.50	3.44	neg	neg	36
3.55	3.78	pos	pos	32
3.57	4.05	neg	neg	1
3.68	3.09	pos	pos	18
3.72	3.99	pos	pos	22
3.87	4.46	pos	pos	11
4.03	4.68	pos	pos	8
4.21	4.35	pos	pos	33
4.33	4.92	pos	pos	12
4.34	5.12	pos	pos	7
4.66	4.74	pos	pos	14
4.71	5.27	pos	pos	6
4.74	4.80	pos	pos	29
4.95	4.92	pos	pos	20
5.17	5.63	pos	pos	24
5.20	5.67	pos	pos	4
5.57	5.75	pos	pos	19
5.58	6.11	pos	pos	30
5.64	5.85	pos	pos	2
5.73	5.88	pos	pos	31
6.29	6.34	pos	pos	40
6.30	6.70	pos	pos	3
6.31	6.60	pos	pos	13
6.40	6.51	pos	pos	17
6.41	6.34	pos	pos	39
6.74	6.30	pos	pos	15



2nd phase DBS Evaluation

- We have planned a 2nd phase evaluation for DBS where the results obtained from the samples extracted from DBS will be compared with the gold standard tests material (whole blood) in infants between 0-18 months old. We will calculate the performance parameters such as sensitivity, specificity, NVP, and PPV.
- We have engaged 3 clinics in Kigali which normally send samples for DNA PCR for NRL.
- The whole blood sample will be tested with Amplicor v 1.5 (Gold Standard) and the results returned to the patients. The sample left over utilized to generate 5 DBS spots. The DBS will be preserved for at least 7 days at room temperature before testing.

2nd Phase Protocol in Rwanda



Results

- Analyzing the results from 345 samples we could find 54 positive and 291 negative samples. The table below shows all parameters of this evaluation. It is clear the good sensitivity (98.02%), specificity (100%), NVP, and PPV of DBS results when compared with GS test.
- In all samples analyzed we could find 1 false negative sample (#406) which will be further analyzed for clarification.



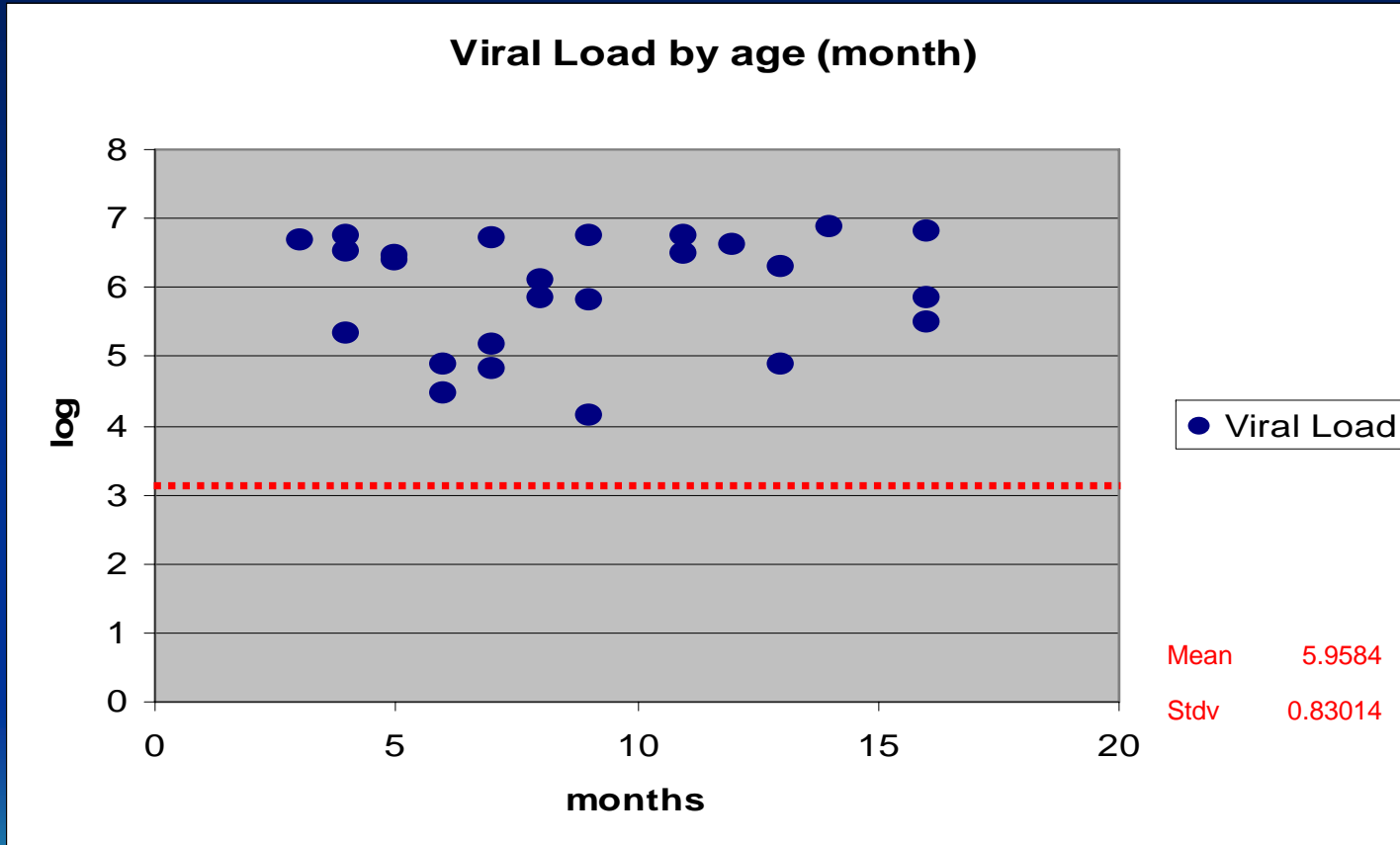
Results 2nd phase DBS Evaluation

Condition		Total S	Present
	Absent		
Test Positive	0	54	54
Test Negative	290	1	291
Totals	290	55	345

Data Analysis

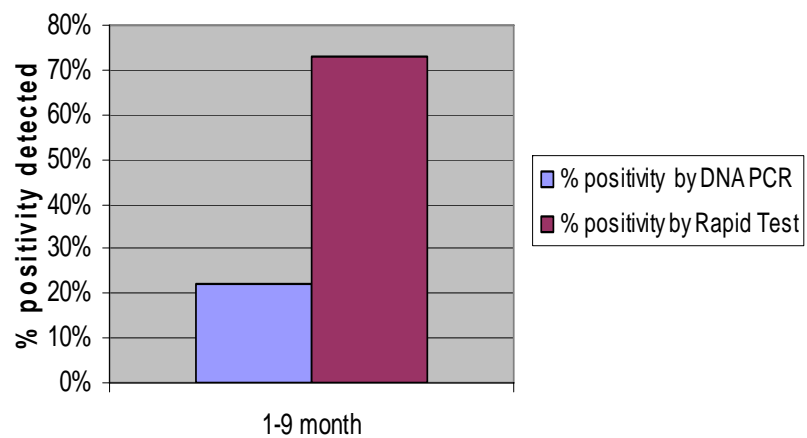
	Estimate d Value	95% Confidence Interval	
		Lower Limit	Upper Limit
Prevalence	0.169435	0.129806	0.217719
Sensitivity	0.980392	0.882072	0.998976
Specificity	1	0.981136	1
For any particular test result, the probability that it will be:			
Positive	0.166113	0.126854	0.214115
Negative	0.833887	0.785885	0.873146
For any particular positive test result, the probability that it is:			
True Positive	1	0.911125	1
False Positive	0	0	0.088875
For any particular negative test result, the probability that it is:			
True Negative	0.996016	0.974535	0.999792
False Negative	0.003984	0.000208	0.025465
likelihood Ratios: [C] = conventional [W] = weighted by prevalence			
Positive [C]	Infinity	NaN	Infinity
Negative [C]	0.019608	0.002816	0.136535
Positive [W]	Infinity	NaN	NaN
Negative [W]	0.004	0.000566	0.028286

Viral Load

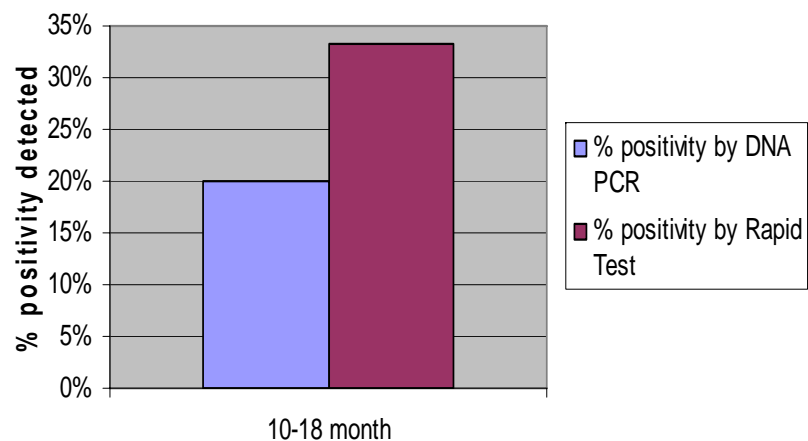


Serology

DNA PCR / RAPID TEST



DNA PCR/RAPID TEST



Serology

Data 0- 9 months

	Estimated	95% Confidence Interval	
	Value	Lower Limit	Upper Limit
Prevalence	0.19481	0.11662	0.30404
Sensitivity	1	0.74653	1
Specificity	0.32258	0.21266	0.45459
For any particular test result, the probability that it will be:			
Positive	0.74026	0.62567	0.83055
Negative	0.25974	0.16945	0.37433
For any particular positive test result, the probability that it is:			
True Positive	0.26316	0.15937	0.39906
False Positive	0.73684	0.60094	0.84063
For any particular negative test result, the probability that it is:			
True Negative	1	0.79955	1
False Negative	0	0	0.20045

Data 10-18 months

	Estimated	95% Confidence Interval	
	Value	Lower Limit	Upper Limit
Prevalence	0.1395	0.058	0.2863
Sensitivity	1	0.5168	1
Specificity	0.7297	0.5561	0.8563
For any particular test result, the probability that it will be:			
Positive	0.37209	0.23394	0.53284
Negative	0.62791	0.46716	0.76607
For any particular positive test result, the probability that it is:			
True Positive	0.375	0.16284	0.64126
False Positive	0.625	0.35874	0.83716
For any particular negative test result, the probability that it is:			
True Negative	1	0.84496	1
False Negative	0	0	0.15504

Conclusion

- The DBS proved to be a reliable way to collect and transport infant samples. The preliminary analysis of test results from the 2nd phase shown similar performance when the results are compared with DNA extracted from whole blood (GS). Taking all in account , the DBS is a nice tool to expand the access for PCR test in exposed children in Rwanda.
- One lab and one tech working full time can test 11000 samples per year.



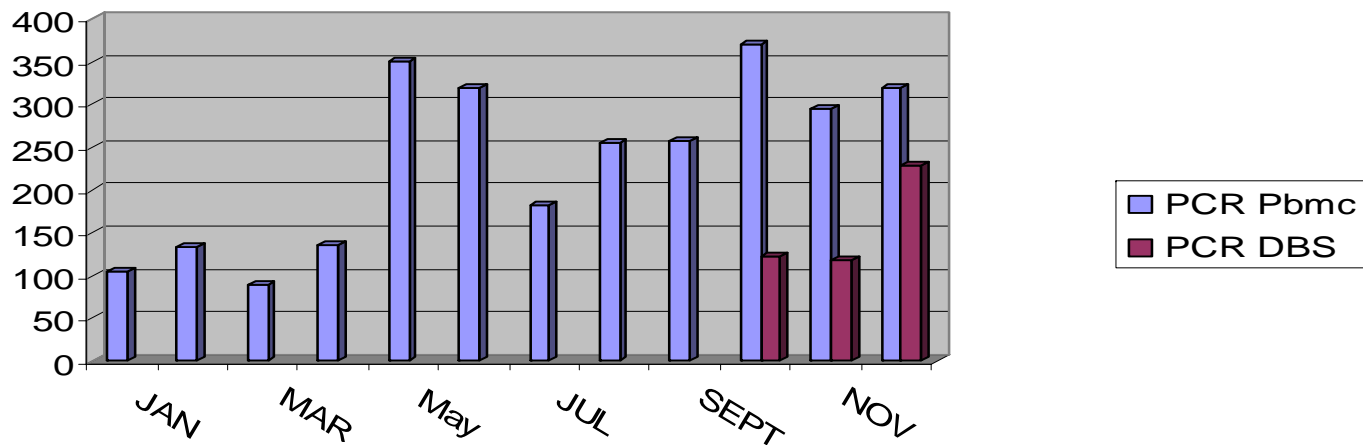
Monitoring the implementation of DBS in 5 HC in Kigali

- 5 sites were chose in Kigali to evaluate the implementation of DBS
- October to December
- Analyzing all logistics aspects involved
- So far 500 DBS DNA PCR from Infants exposed have been made.



DNA PCR Infant Diagnosis NRL Rwanda

PCR for HIV Infant Dx
Pbmc and DBS
In Rwanda 2005



The Whole Team

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THANK YOU

AMAKURU

GRACIAS

