RAPID HIV TESTING TECHNIQUES

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Rapid testing has become a popular topic over the last year or two. I would like to provide an overview of the types of activities CDC has been involved in over the last ten years with rapid HIV diagnostics. We have looked at how well these assays perform, not only individually but also in the context of algorithmic combinations.

I would like to acknowledge several people who have been involved with our work over the years. These professionals work internationally and within our own laboratories. We have worked extensively with the Honduran Ministry of Health and I will share some of the data we obtained through that collaboration. The Ministries of Health in Trinidad and Tobago, and the Bahamas, the HIV AIDS Collaboration in Thailand, as well as the Uganda Viral Research Institute have also collaborated with us. Drs. Parekh and Branson have provided abundant guidance on these tests and set up many of these projects.

In order to be assured that our findings are based on very hard science, we must undertake a close review of all the things we are going to be doing, and then, of course, record accurate results.

(Slide 1) There have been three basic types of formats that have been developed. The first is the flow through device, where the reagents pass through a wicking column. There are also agglutination tests. Both of these were developed fairly early in the rapid HIV arena. The one that has arrived on the scene most recently is the dip stick format. We will try and go through all of these and give you some of our findings regarding performance.

(Slide 2) The flow through device is made of hard plastic with wicking material underneath it. It has a membrane situated within the device on which the HIV antigens are placed. All the reagents and the sample are simply run through this device, with the sample antibodies binding to correlating HIV antigens which are on the membrane. These in turn bind a conjugate and a precipitable substrate. There are a variety of tests that have been developed along the lines of these formats. Some of these, like the Multi spot, HIV-CHEK AND Testpak, have been tested for a long period of time, and have proven to be quite rugged assays.

Slide 3 shows the agglutination formats. One of this type that came on the scene very early was the Recombigen, but it did not work particularly well. This was originally licensed, but it has now been withdrawn from the market. Other tests are based on latex beads or gel particles on which antigens are placed. Antibodies, in the presence of the HIV antigens, will cause cross linkage and clumping will result.

(Slide 4) This particular device is called a Capillus and is fairly new. It works using the same principle, but it uses a device which allows an indicator to migrate downward. There are some constrictions in the device which force the particles together and improve the binding process.

The Serodia and Simpli-Red are two products that have been used fairly extensively in Asia. The Serodia now has been modified and Simpli-Red was renamed Simpli-Dry because it lyophilizes all of the reagent.

The last format which has been quite prominent in the last few years are the dip stick devices which impregnate all of the reagents in the lower part of the device with an absorbent pad. The dipstick is placed in contact with the sample. As the sample begins its migration through this device, it picks up all the reagents. The antibodies present will bind to the HIV antigen. An IgG control line is added to insure that the test specimen has been added. A second will develop if HIV antibodies are present. These are immersed in a colloidal suspension e.g., colloidal gold which will bind to the human IgG if it is present. In looking at new devices, it is always important to determine our goals before we jump into a situation that we would rather not be in.

(Slide 6) In the early 1990's, we planned a study with the Hondurans to look at the basic performance of the rapid products that were available at that time. This was to be a three phase study. First, we looked at a retrospective evaluation of the various tests and how those tests were performing. We followed this with a second, prospective evaluation in regional hospitals. We attempted to enroll 100 positives and 200 negatives at each site. The performance of these products was evaluated in a third phase conducted at health clinics in Honduras. Later, we would place the products there as a routine test to see how well these assays performed relative to our EIA/Western blot algorithms. The tests that were available at that time are listed. The Retrocell was also included in the study. Although this is not a rapid test, it is a very simple one, one that the Hondurans were using routinely and were very comfortable with.

(Slide 7) In looking at the performance of the assays in phase 1, you can see the sensitivity of all of the assays were very good. The specificity was lacking in some of the tests, particularly in the Serodia. This probably had to do with the subjective review of this particular test. Performance may have improved with more testing carried out by the operators. Certainly these tests performed very well in comparison to the EIA and Western blot in the retrospective phase of the study.

We could not continue with all of the tests in the second phase, but several moved forward. During phase 2, 100 positives and 200 negatives were collected at three sites. I will not show you all of these results, but the bottom line was to be determined by field performance. After phase 2, another set of evaluations were undertaken and selection of the products that were put into phase 3. (Slide 8) These are the assays that were taken to phase 3: Retrocell, HIV-chek, and the Genie, which is now known as the Multispot. Of course, HIV-chek and Genie both have been modified since this initial study, but even these results still are very good.

In showing the field sensitivity of the immunoassays, this is what was actually reported at the site of testing. If the discordant samples were retested, most of them came up positive. In this particular case, all were reported positive. However, we were interested in the functioning of the algorithm, as well as the test itself. We chose to proceed with these categories of reporting.

As the study began, the World Health Organization (WHO) proposed some testing strategies, not only for rapid tests, but for all HIV assays. These were to be tailored to what you were attempting to accomplish with your testing. (Slide 9) Using these three different strategies, depending on your approach, blood donation would require only a single assay. If it were positive it would be removed. If prevalence was less than ten percent, you would have to go into two assays. An asymptomatic infection then would require three assays that would be positive.

We tried to put our study tests into this type of context to see how well they actually performed, and to determine if we could use these assays in

combination to get an accurate result. The only thing we knew about the individuals that came up for testing was the purpose for them being there. During this time in Honduras, the only reason for testing blood donations was suspicion of HIV positivity.

(Slides 10 and 11) We were able to break this population down into two categories. One was a very low prevalence group, and there were thirteen positives in this group. All of the assay combinations tested with excellent sensitivity and specificity. In the context of the higher prevalence groups, 30% of the six sites indicated testing performance was good but not comparable to EIA and Western blot. There was still an effective way of testing, particularly in these rural areas where high technology was not available. Actually, Honduras chose to stay with this particular combination. They were comfortable with the Retrocell plus HIV-CHEK, and they are using this method today in the outlying areas.

(Slides 12-14) We did a very similar study using the same types of products in the Caribbean with a much larger sample set of 4,400 specimens. The performance of these assays again proved to be good. The third assay was one that was on an ELISA plate and was read visually. Thus, the sensitivity and specificity suffered from the subjective evaluation of the product. If you look at the Genie and HIV-CHEK in combination, there was very good performance with a 99.8% sensitivity and 100% specificity.

It is clear these tests can be formulated quite well to provide a good answer. One of the problems with these types of tests is that they are still relatively expensive. The least expensive around $3.00 and they can range up to $10.00. There is still room for improvement in reducing the cost and applying these tests in a variety of settings. (Slide 15) Cost is what precipitated the development of the new types of rapid HIV tests. Most of these are, once again, the strip-based type of assays. Some of these, the Genie II and Quix are scaled down models of the flow through devices.

(Slide 16) This is a schematic of the Uni-Gold test. The sample is simply wicked through the device, which is a strip encased in a piece of plastic. The procedure takes about five or ten minutes to run to completion. Many of these have been formulated for use with whole blood. Also, on the capillary end, you simply lance a finger and the sample will be absorbed onto the test strip. The buffer tube is placed down into the sample. The sample is absorbed and you get an answer just as you would see on the strips. It is a very nice, single-use type of device.

(Slide 17) This is the most recent device from Abbott. It is called the Determine. It can also detect subtype O. You can see this is simply a strip with a place for the sample to be added.

All of these products are now $3.00 or less, which has greatly assisted those performing these assays in developing countries. We examined the basic laboratory and field performance of these assays to determine how well they were able to detect low titer antibody. Certainly these are not going to be as good as the mechanized versions, but determining how well they can detect low titer antibody in seroconverters, and if they can detect antibodies to the various strains, is crucial.

Slide 18 shows the Sero-Strip which is similar to the Hema-Strip. It is not encased in plastic and has been made to use with serum. We observed very good sensitivity and specificity in this particular population of approximately 2,000 specimens.

(Slide 19) The Capillus is a latex agglutination assay device. Although a much smaller number of positives were observed in this study, the results indicated that the test detected all of the positives. I think this is important to stress when

looking at how well these devices perform in different environments and situations. Most of these samples were North American sera. I will also report how these devices perform in other areas of the world. It is important to evaluate these particular products in the area that you are going to use them.

Before I look at detection of sera collected from individuals infected with different HIV subtypes, I will mention what we did with seroconversion panels. We took 10 seroconversion panels and tested each panel with each of the six rapid assays. All we knew about the seroconversion panels was the date of the initial collection and each of the subsequent collections; from which we could determine the number of days for the detection of a positive specimen. We took the data for each of the panels and averaged the number of days required for identifying the initial positive for all of the rapid tests. The Abbott third generation test detected the initial positive specimens from the 10 seroconversion panels in an average of 31 days. Western blot positivity was not observed in the panels until about 39 days. All of the six rapid tests that we used were statistically equivalent to the detection of the first positive specimen by Western blot. Thus, the rapid assays performed as good as Western blot, but were slightly less effective than the Abbott EIA in the detection of early seroconversion.

(Slide 20) When looking at the different HIV strains, we selected a panel from a variety of locations around the world. We selected representative specimens from individuals that were infected with the different HIV subtypes. I show the data using the Sero-Strip assay and it is representative of the data derived for the other assays. All of the sera from the subtype panel for Group M were detected by the Sero-Strip device and by most of the other rapid assays. The only samples that were difficult to detect were the HIV Group O specimens. Some of the assays, such as Capillus, missed all of the Group O samples. I think this is probably due to the low concentration of HIV-specific antibodies induced by the Group O specimens which will react with antigens found in the subtype B strains. Antigens of the subtype B isolates were used primarily in the development of the rapid assays. Assay format is another issue affecting the ability of the rapid assays to detect HIV antibodies. The RTD rapid assay, manufactured by the same company that produces the Capillus and which uses the same antigen set as Capillus, detected all of the Group O isolates using the flow through, antibody-enzyme conjugate design.

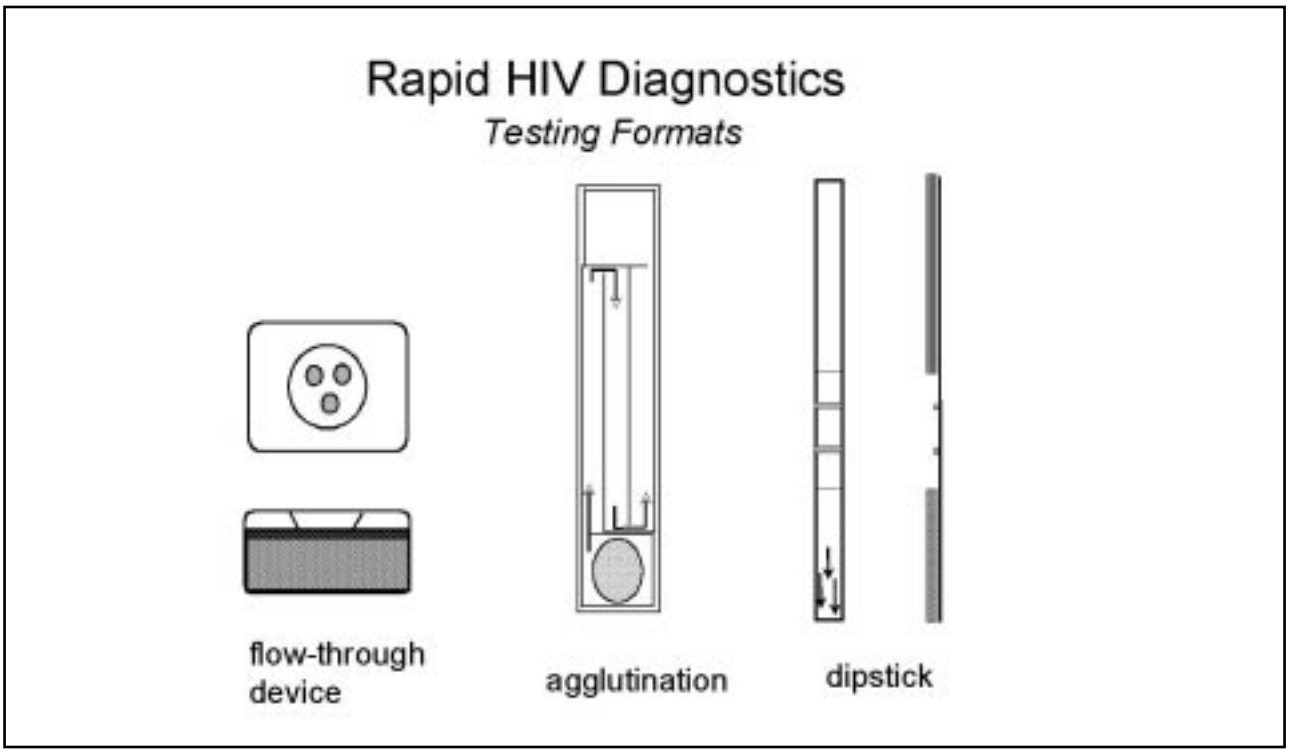
(Slide 21-23) Now we will examine how these assays perform in other areas of the world. In these next few slides are data obtained from work done in Uganda with approximately 250 specimens. The sensitivity of the Sero-Strip was only 98%; it performed better amongst the North American specimens. Sero-Card is another system which is similar to a downsized flow-through device. It performed very well in this setting. Capillus rated higher in terms of sensitivity, but had some difficulty with specificity. When these data were put together in different types of algorithms, they did not function as well as predicted. With large data sets, some of the sensitivity and specificity outcomes were not as good as had been hoped for. That is why it is important that we look at these particular devices in each area where they are potentially going to be used.

(Slide 24) This slide contains some very recent data. We have tried to get a study set up to look at some of the new whole blood test kits. Initial testing was performed with serum just to give us a general idea of how well the assay was performing. We plan to evaluate whole blood testing in the laboratory, and eventually in a clinic setting. The Uni-Gold and the Capillus seem to perform very well. The Hema-Strip missed four of these particular specimens. The Western blot patterns of the missed samples show that they

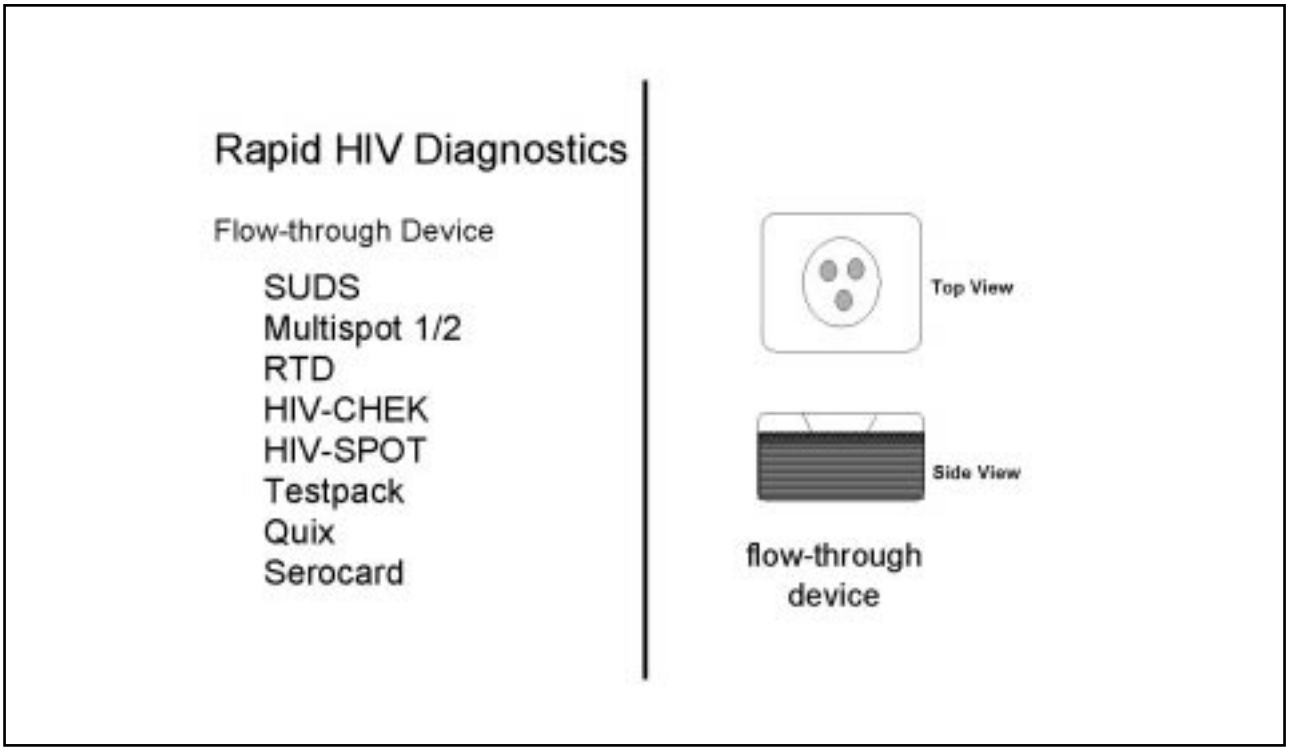
were all essentially the same type of specimens, i.e., these were very low titer antibodies and probably represent seroconversion. That is what we saw earlier with seroconversion panels. There may be a slight delay in picking these up with certain rapid test devices.

(Slide 25) In summary, these assays are comparable to the standard EIA we are using presently. But, are certainly not as good as the assays that are currently being developed. Once the third generation Abbott test became available, we started to have the ability to separate and detect antibodies earlier compared to these rapid diagnostic tests. Using rapid tests in combination certainly affords us an avenue, in certain situations, to obtain very good answers. However, once again, with the improvements in the standard EIA and Western blot, we are not going to attain comparability. However, it will still be a very good way of doing testing in certain settings. Although I did not mention the adaptability of these types of products to whole blood and to oral transudate fluids, there are rapid testing products that are being developed for use with oral fluids.

Slide 1



Slide 2

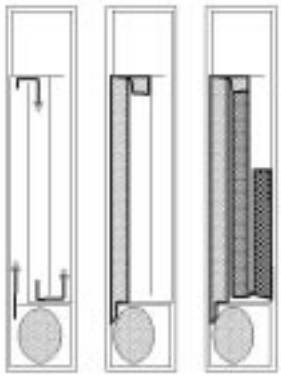


Slide 3

Rapid HIV Diagnostics

Agglutination Device

Recombigen
Serodia
Capillus
Simpli-Red

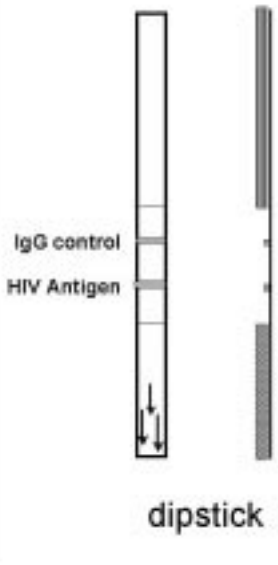


Slide 4

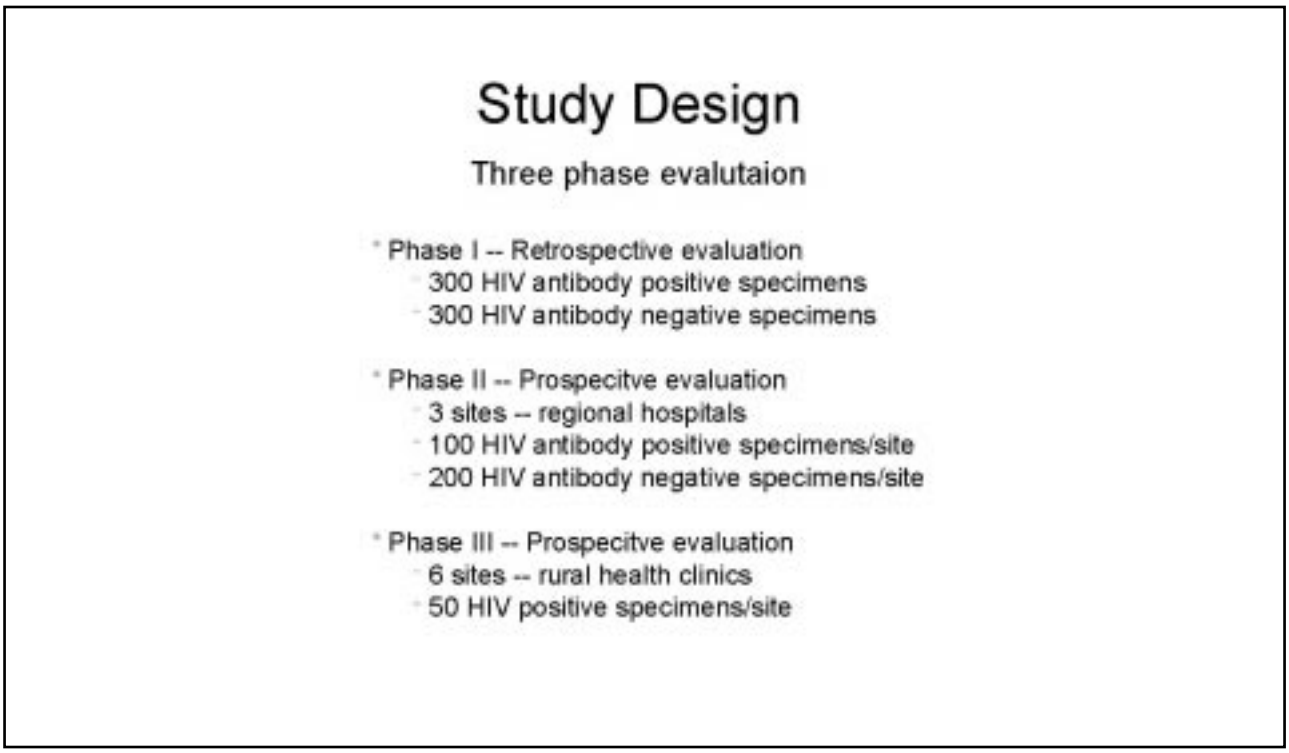
Rapid HIV Diagnostics

Dipstick or
Capillary Flow Device

Sero-Strip
Hema-Strip
Uni-Gold
Determine



Slide 5



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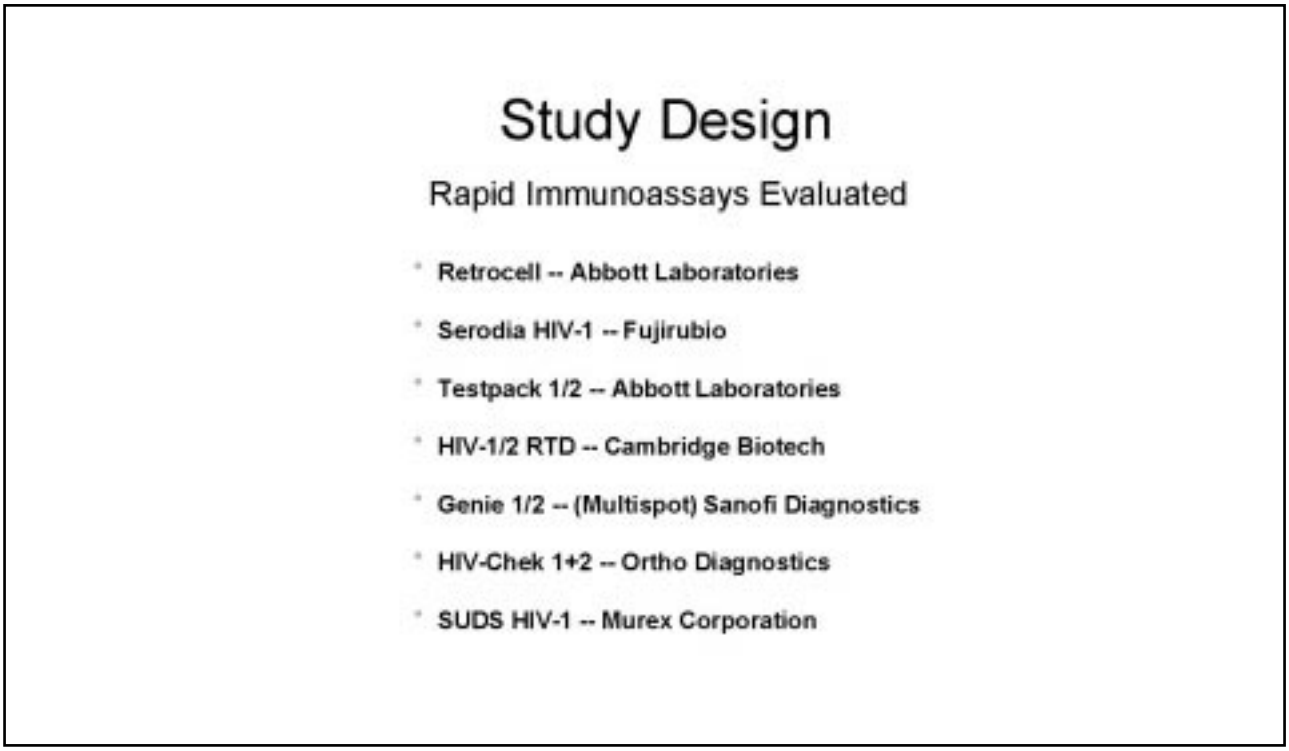


Slide 7

HIV Rapid Immunoassays

Performance -- Phase I

Rapid Assay	Sensitivity (%)	Specificity (%)
Retrocell	100	98.6
HIV-Chek	99.7	100
Genie	100	100
Testpack	100	98.9
SUDS	99.3	96.3
Serodia	100	92.8
RTD	100	97.5

N=600

Slide 8

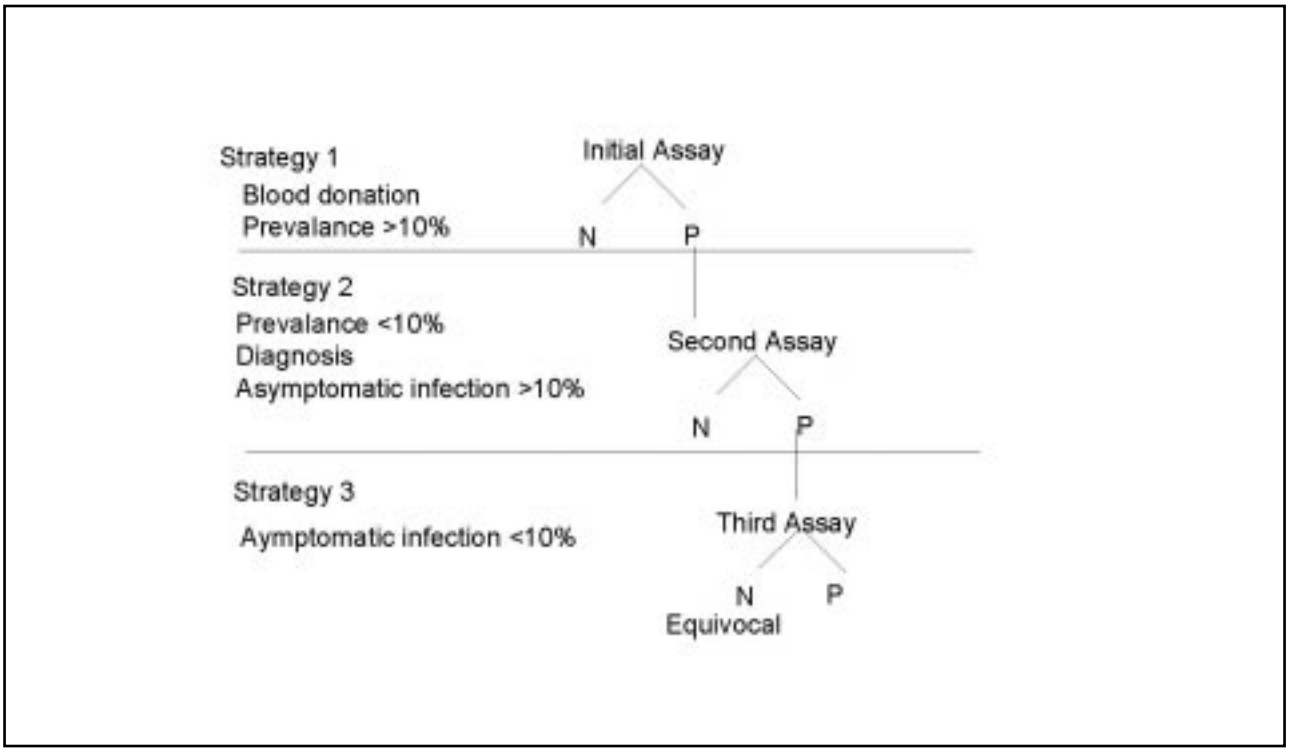
HIV Rapid Immunoassays

Phase III -- Performance

Rapid Assay	Field Sensitivity (%)	Corrected Sensitivity (%)	Specificity (%)
Retrocell	**99.3**	**100**	**99.8**
HIV-Chek	**99.3**	**100**	**99.9**
Genie	**99.3**	**100**	**100**

N=1255

Slide 9



Slide 10

HIV Rapid Immunoassay Algorithms

WHO Strategies -- Low prevalance (1.5%)

Algorithm	Sensitivity	Specificity	PPV	NPV
Retrocell + HIV-Chek	100	100	100	100
Retrocell + Genie	100	100	100	100
HIV-Chek + Genie	100	100	100	100

N=857 PPV= Positive Predicitive Value, NPV= Negative Predicitive Value

Slide 11

HIV Rapid Immunoassay Algorithms

WHO Strategies -- High Prevalance (30.5%)

Algorithm	Sensitivity	Specificity	PPV	NPV
Retrocell + HIV-Chek	99.2	100	100	99.6
Retrocell + Genie	99.2	100	100	99.6
HIV-Chek + Genie	98.4	100	100	99.3

N=1255

Slide 12

Rapid HIV Testing in the Caribbean

Centers for Disease Control and Prevention
Caribbean Epidemiology Centre
Ministry of Health and Environment, Nassau
Bahamas
Ministry of Health, Trinidad and Tobago

Slide 13

Performance of Rapid HIV Immunoassays

Assay	Sensitivity (%)	Specificity (%)
Genie	100	99.9
HIV-Chek	99.8	99.3
SID peptide EIA	96.9	98.8

N=4448

Slide 14

Performance of HIV Rapid Test Algorithms

Algorithm	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Genie + HIV-Chek	99.8	100	100	99.97

N=4448

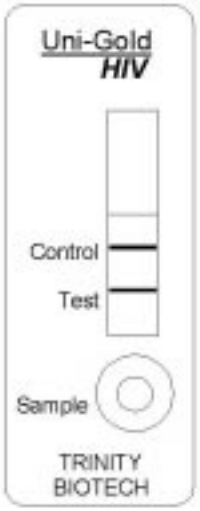
Slide 15

New Generation Rapid HIV Antibody Tests

- Uni-Gold
- Determine
- Sero-strip
- Hema-strip
- Saliva-strip
- Simpli-dry
- Genie II
- Quix

Slide 16

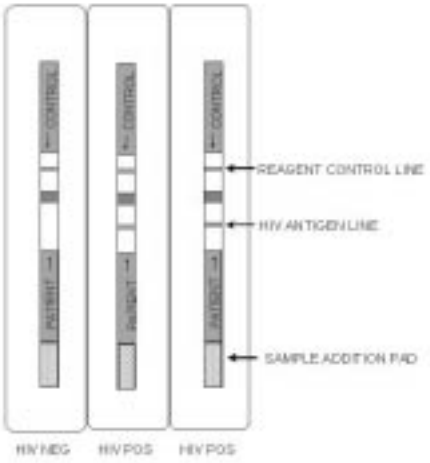
Uni-Gold HIV



Slide 17

Determine HIV-1/2 Rapid Test

Abbott Laboratories



Slide 18

Sero-Strip HIV-1/2 Performance

N=1917

		Sero-Strip HIV-1/2 P	Sero-Strip HIV-1/2 N
WB	P	908	6
	N	1	1002

Sensitivity = 99.2%
Specificity = 99.9%

Slide 19

Sero-Strip HIV-1/2 Performance

N=1754

Capillus HIV-1/2

		P	N
WB	**P**	**210**	**0**
	N	**1**	**1543**

Sensitivity = 100%
Specificity = 99.9%

Slide 20

Ability of Dipstick Rapid Test to Detect Antibodies Induced by HIV Variants

HIV Specimens	Number	Sero-Strip Results P	Sero-Strip Results N
A	40	40	0
B	47	47	0
C	8	8	0
D	28	28	0
E	42	42	0
F	7	7	0
O	3	2	1
HIV-2	25	25	0
Negative	69	0	69

Slide 21

Performance of Rapid HIV Immunoassays in Uganda

Uganda Virus Research Institute
AIDS Information Center, Kampala, Uganda
Centers for Disease Control and Prevention

Slide 22

Rapid HIV Immunoassay Performance
Uganda

Rapid Immunoassay	Sensitivity (%)	Specificity (%)
Sero-Strip HIV 1/2	98.4	100
SeroCard HIV	100	99.5
Capillus HIV-1/2	100	97.3

N=248

Slide 23

Rapid HIV Immunoassays

Algorithm Performance

Algorithm	Sensitivity (%)	Specificity (%)
Sero-Strip + SeroCard	98.4	100
Sero-Strip + Capillus	98.4	100
Sero-Card + Sero-Strip	98.4	100
SeroCard + Capillus	100	100
Capillus + Sero-Strip	98.4	100
Capillus + SeroCard	100	100

N=248

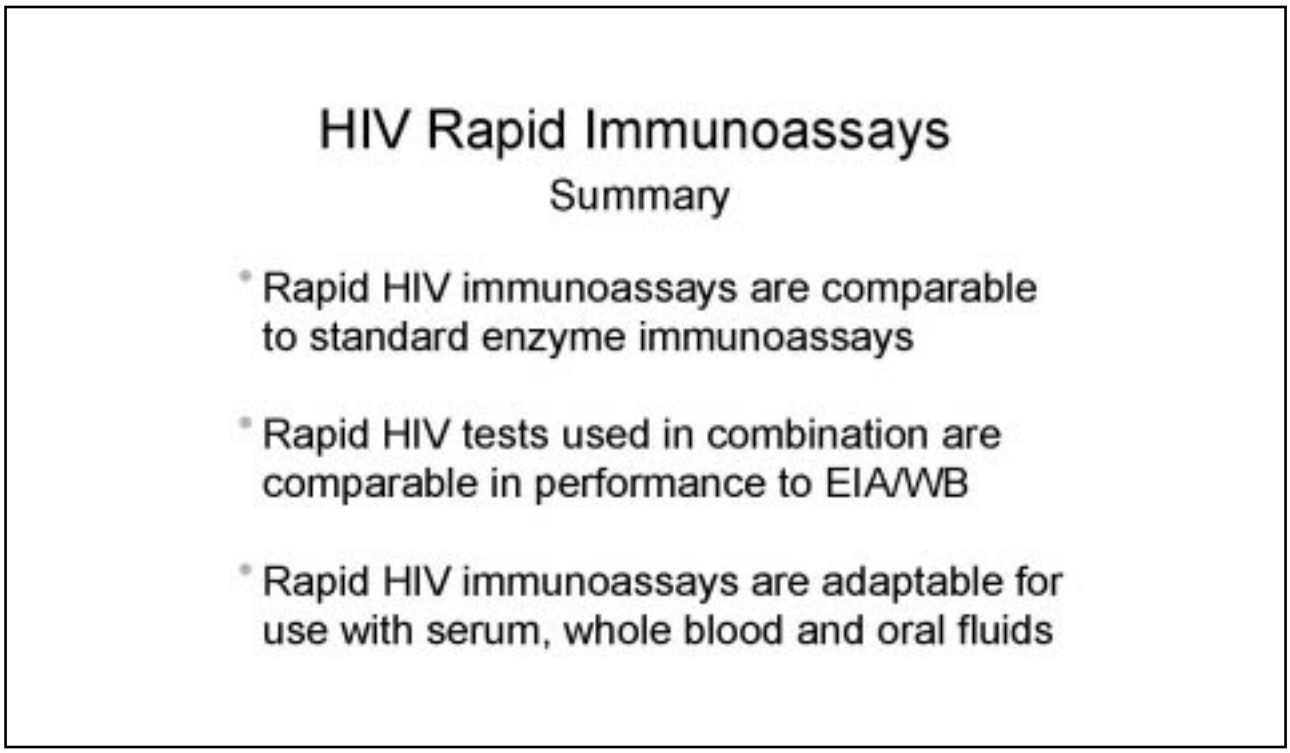
Slide 24

Performance of HIV Rapid Diagnostics HOT DATA

	Hema-Strip		Uni-Gold		Capillus	
	P	N	P	N	P	N
WB P	127	4	130	1	129	2
WB N	1	150	7	144	0	151

N=282

Slide 25


HIV Rapid Immunoassays
Summary
Rapid HIV immunoassays are comparable to standard enzyme immunoassays
Rapid HIV tests used in combination are comparable in performance to EIA/WB
Rapid HIV immunoassays are adaptable for use with serum, whole blood and oral fluids