

Development of the VITROS HIV Combo assay

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Product Development

Ortho Clinical Diagnostics
a *Johnson & Johnson* company



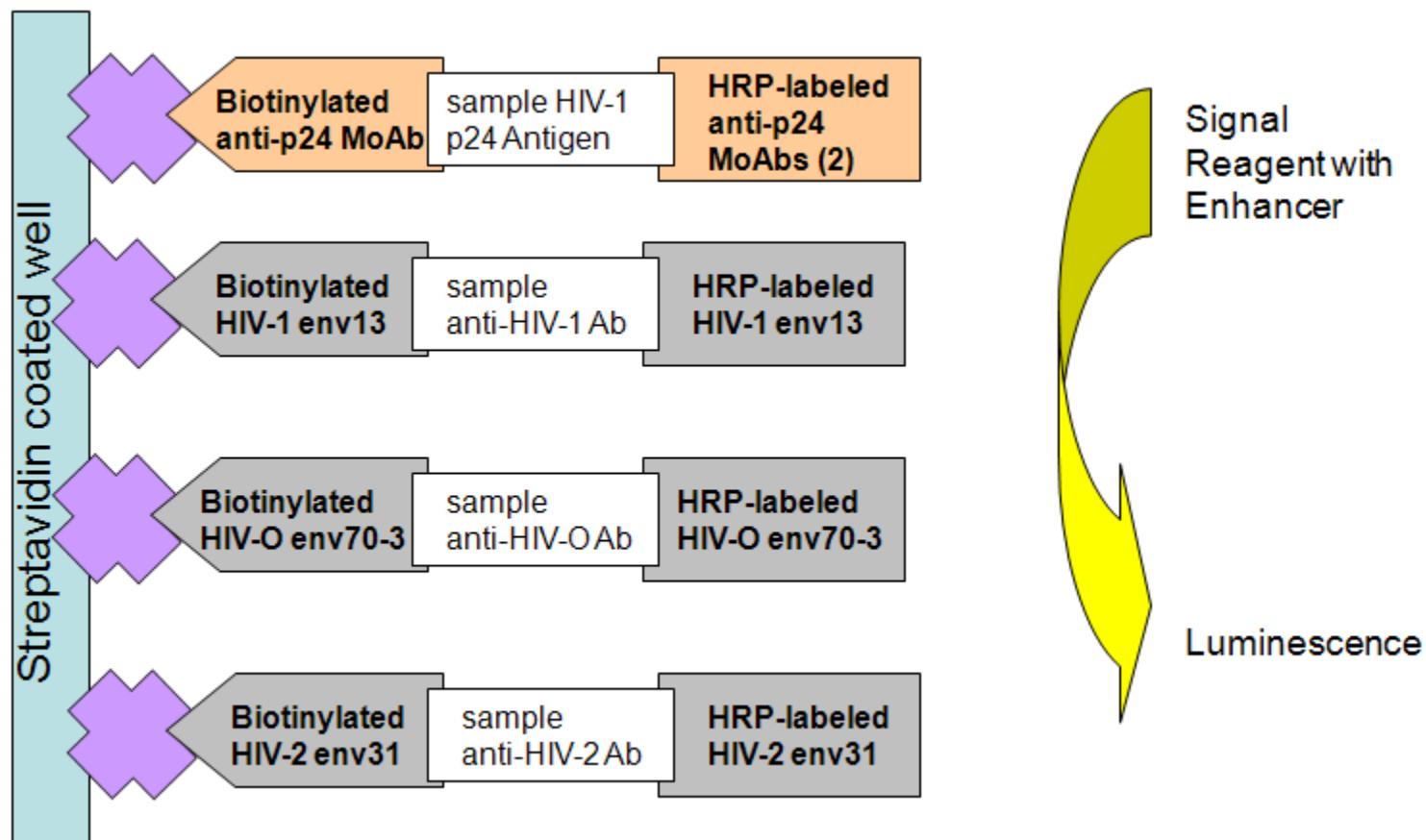
Development of the VITROS HIV Combo assay

- We are currently developing a 4th generation HIV assay for use on any of the VITROS Systems capable of running Microwell assays. These Systems are Random Access.
- The development is a close collaboration between scientists at Novartis Vaccines and Diagnostics and Ortho Clinical Diagnostics
- The VITROS HIV Combo assay is currently under development

Introduction

- Assay design and timing
- Antibody sensitivity
- Seroconversion sensitivity
- P24 antigen sensitivity – against recognized standards
- Detection of different antigen types
- Specificity – particularly against challenging clinical samples
- System features that benefit HIV testing

Assay design and timing



Two-step assay with TFR 48 minutes,

Antibody Sensitivity

Samples	VITROS HIV Combo S/CO	Market Leading HIV Ag/Ab Combo Assay S/CO
HIV-1 Ab dilution samples		
96938 1:20K	12.90	7.10
96938 1:40K	6.40	3.70
96938 1:80K	3.80	1.90
96938 1:160K	1.60	0.96
96938 1:320K	0.70	0.60
96938 1:640K	0.50	0.30
9031-17 1:16	5.20	0.70
9031-17 1:32	1.80	0.50
9031-17 1:64	0.90	0.30
9031-17 1:128	0.70	0.30
HIV-2 Ab dilution samples		
91181 1:5K	2.90	2.60
91181 1:10K	1.30	1.10
91181 1:20K	0.60	0.70
HIV-1 Group O Ab dilution samples		
ODS-002 1:100	17.2	12.70
ODS-002 1:200	10.2	7.70
ODS-002 1:400	5.60	5.30
ODS-002 1:800	3.10	3.90
ODS-002 1:1600	1.60	2.60
ODS-002 1:3200	0.90	1.60

Seroconversion sensitivity

Seroconversion Panel	Bleed day of 1st positive result		Difference (days)
	VITROS HIV Combo	VITROS Anti-HIV 1+2	
SeraCare PRB939	16	25	9
SeraCare PRB943	7	19	12
SeraCare PRB950	18	28	10
SeraCare PRB951	8	15	7
SeraCare PRB954	17	21	4
SeraCare PRB957	23	23	0
SeraCare PRB965	5	12	7
Zeptometrix 6243	36	38	2
Zeptometrix 6244	33	38	5
Zeptometrix 9018	25	32	7
Zeptometrix 9020	90	97	7
Zeptometrix 9032	22	24	2
Average earlier detection			6

HIV-1 p24 Ag sensitivity

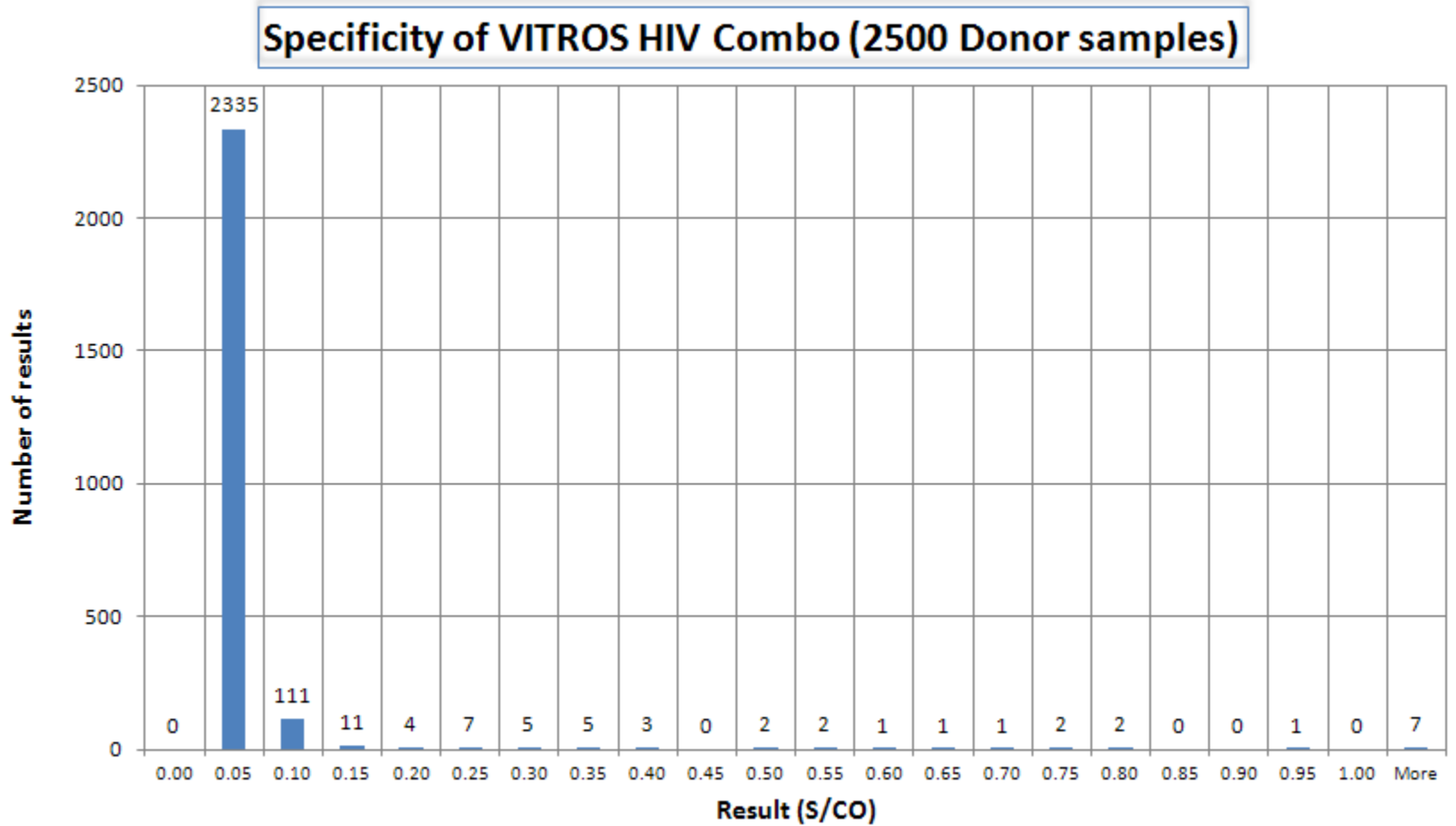
Samples	VITROS HIV Combo S/CO	Market Leading HIV Ag/Ab Combo Assay S/CO
AFSSAPS p24 standard (pg/mL)		
200	15.75	9.6
100	7.50	4.5
50.0	3.15	2.5
25.0	1.40	1.3
12.5	0.65	0.8
6.25	0.15	0.5
Sensitivity at S/CO of 1.0 in pg/mL	18.40	17.8
WHO p24 Standard (IU/mL)		
10.0	14.00	9.1
5.00	7.20	4.6
2.50	3.40	2.4
1.25	1.70	1.2
0.625	0.80	0.7
0.313	0.50	0.4
Sensitivity at S/CO of 1.0 in IU/mL	0.69	0.98

Detection of HIV-1 antigen genotypes

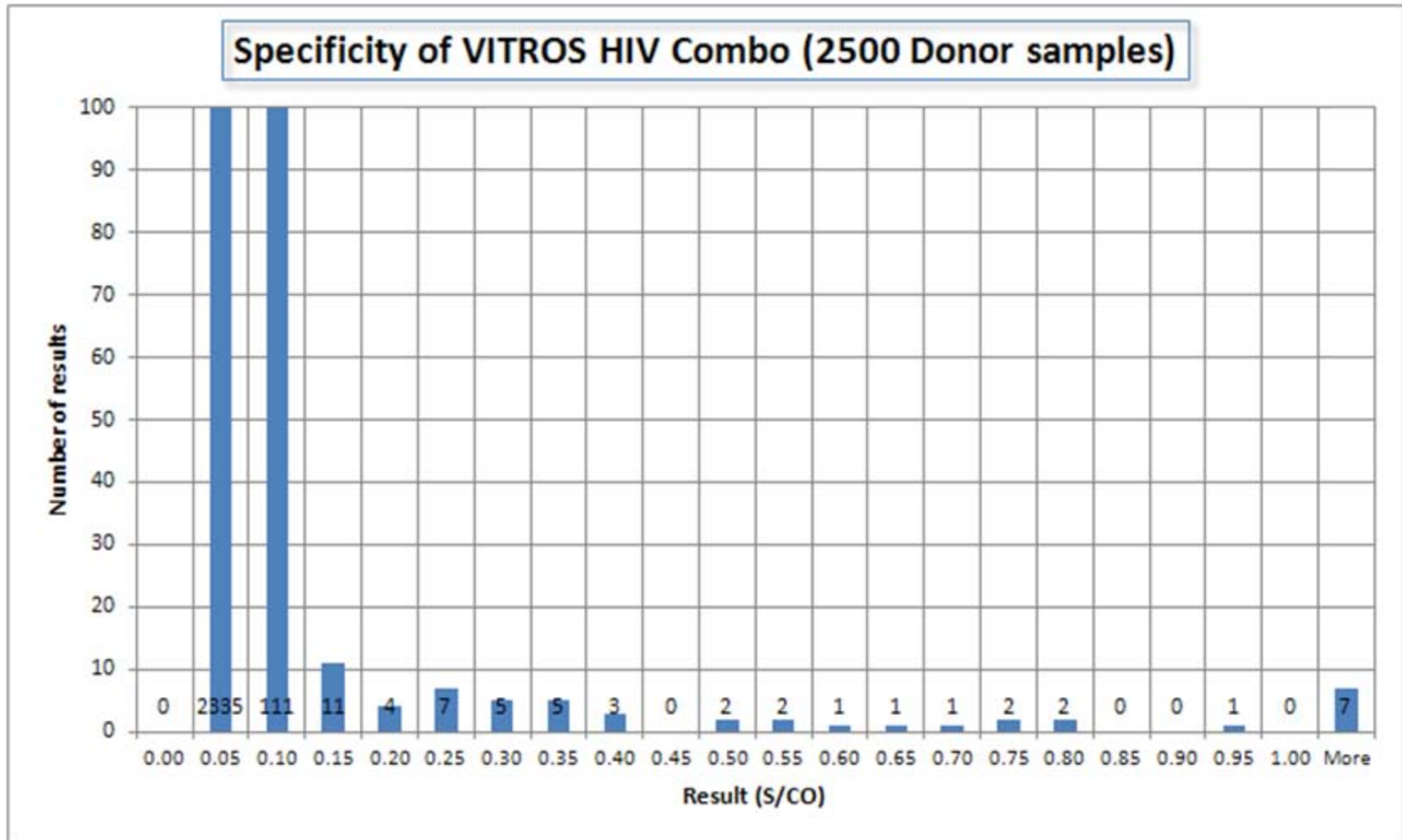
dilutions of HIV-1 Group M & O virus

Sub-type	Isolate ID	Country of Origin	VITROS HIV Combo (S/CO)
HIV-1 Group M Subtype samples			
A	UG273	Uganda	3.90
AG	DJ263	Djibouti	3.60
B	BK132	Thailand	1.40
C	SM145	Somalia	5.40
D	UG274	Uganda	1.90
AE	CM240	Thailand	5.70
F	BZ163	Brazil	3.10
G	BCF-DIOUM	Zaire	3.70
H	BCF-KITA	Zaire	3.50
HIV-1 Group O samples			
O	BCF06	Cameroon	3.60
O	I-2478B	USA	3.60
O	I-2481	Spain	1.70
HIV-2 sample			
A	NIH-Z	Guinea-Bissau	6.20

Specificity



Specificity



Specificity- summary

Statistics for 2500 normal donor samples	
Mean S/CO	0.05
Number of samples > 1.00 S/CO	7
Max S/CO	3.94
% specificity	99.72%
95% Confidence Interval	99.42 to 99.89%

Sample category	Number tested	Number negative	% Specificity
Blood donors	2500	2493	99.72
Clinical	194	194	100.00
Lipid	10	10	100.00
RF/HAMA	31	31	100.00
Pregnancy	28	28	100.00
Cancer	41	41	100.00
Cardiac	35	35	100.00
Thyroid	49	49	100.00

Other considerations

Key focus of assay development activities at OCD is to ensure consistent results lot to lot and instrument to instrument

- **Calibrators** - are optimized to consistently position cut off whilst supporting 28 day calibration interval and excellent long term stability.
- **Coated Wells** - Coating methods optimized to ensure consistent clinical and analytical performance lot-lot.
- **Reagents** - Formulations optimized to ensure consistent clinical and technical performance, a high degree of protection against interference from “problem samples” such as anti-HRP activity, heterophilic antibodies, HAMA and RF.
 - Excellent Sensitivity
 - Excellent Specificity
- **Secure manufacturing processes** with appropriate documentation aligned with J&J and FDA Quality Systems regulations—very successful FDA GMP inspections

VITROS® Systems

System
VITROS® 5600
Integrated



System
VITROS® 3600
Immunodiagnostic



System
VITROS® *ECiQ*
Immunodiagnostic

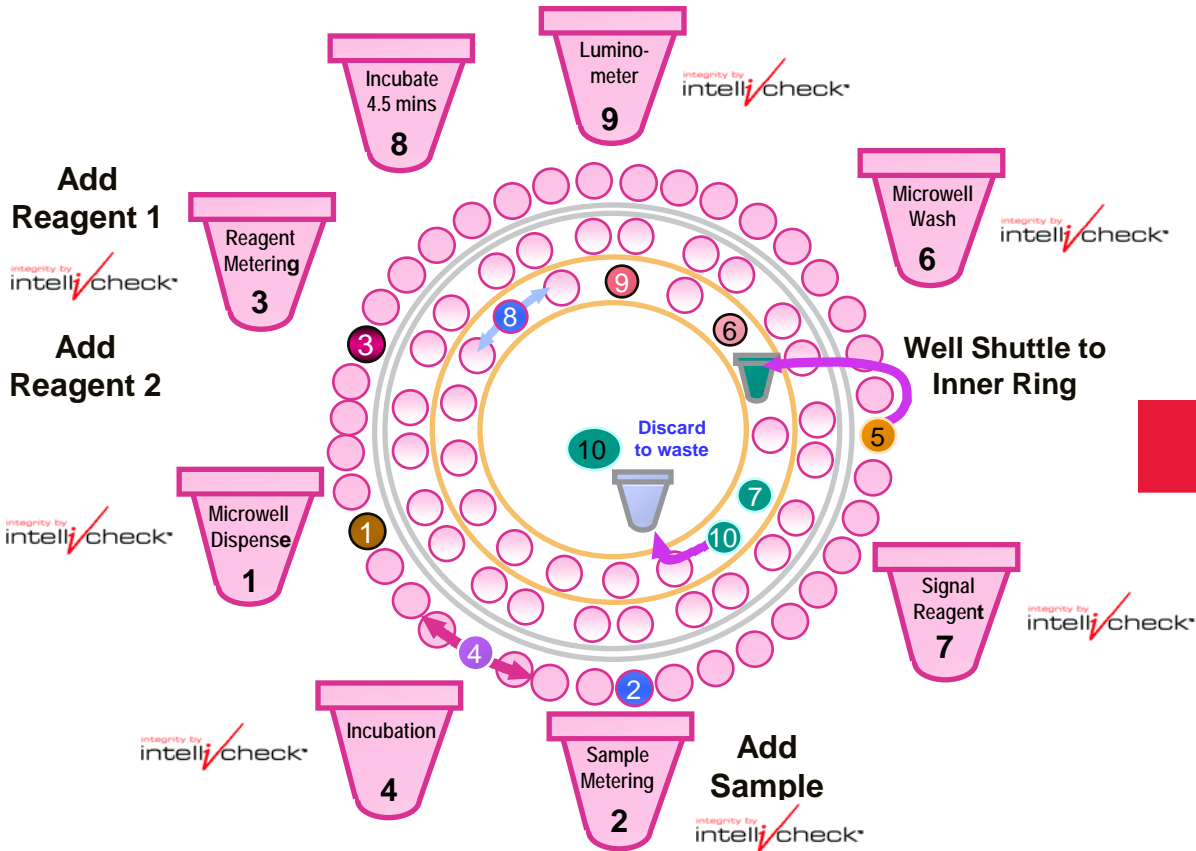


System features that benefit HIV testing

- Disposable tip sample metering
- Clot detection
- Bubble detection
- Short sample detection
- High/low viscosity detection
- Sample + Reagent Verification
- Well Wash Dispense Verification
- Signal Reagent Dispense Verification



Intelligence[®] Technology



IntelliReport

Results Review - IntelliReport

Sample ID: TSH10 Assay: TSH Rep: 2/3 Lot: 1661 Date: 5/8/2007 05:25:47

SAMPLE METERING		REAGENT		WELL WASH VERIFICATION		
Deleted?	Detected?	Plugged Tip:	Detected?	Actual Value:	Target Range:	Absolute Range:
Clot: No	Plugged Tip: No	Bubble: No	Plugged Tip: No	1: 8287	2876-5148	2047-605
Bubble: No	Plugged Tip: No	Short Sample: Yes	Plugged Tip: No	2: 7908	7307-8487	199-6872
Viscosity: No	Plugged Tip: No	Thin layer of fluid: No	Plugged Tip: No	3: 1201	4694-3228	894-944
Mis Exception? Yes	Plugged Tip: No	Viscosity Profile: >12	Plugged Tip: No	4: 2877	2305-6332	7149-6885
Viscosity: >12	Plugged Tip: No		Plugged Tip: No	5: 7062	6944-6965	635-6685
	Plugged Tip: No		Plugged Tip: No	6: 9642	3393-566	520-1474

SAMPLE INDICES

Actual	Upper	HT	Flags
Hemolytic: NA	159	NR	
Interfer: 145	NR		
Turbidity: 46	NR		

LUMINOMETER

Actual Value:	Expected Value:	IntelliCheck Range:
5524	6313	6211 - 6361

SIGNAL REAGENT

Actual Value:	Expected Value:	IntelliCheck Range:
37	33	17-37

Select a button to perform the corresponding function:

Return Previous Result Next Result Print Report Help

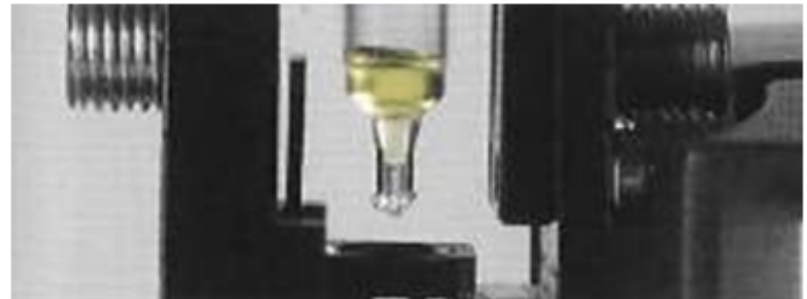
Reports the quality of each result

MicroSensor Technology

– Sample Indices

Microsensor technology detects endogenous interferences

- Haemolysis, Icterus & Turbidity
- Fiber-optic wavelength scan of sample
- Flags affected results
- No reagent or additional sample volume requirements
- No impact on system workflow or turnaround time



MicroSensor™ Technology –
Reduces error, time, labour, and costs associated with visual sample indices interpretation

Conclusions

We are developing a 4th generation HIV assay that delivers the following level of performance:

- Good HIV-1, HIV-2 and HIV-1 subgroup O antibody reactivity
- Seroconversion sensitivity approximately 6 days ahead of antibody only assays
- Excellent antigen sensitivity when measured with recognized P24 standards
- Good detection of different antigen types
- Good specificity in a normal donor population and with challenging clinical samples
- Utilizes system features that benefit HIV testing