Hytest

Building Better HIV Testing

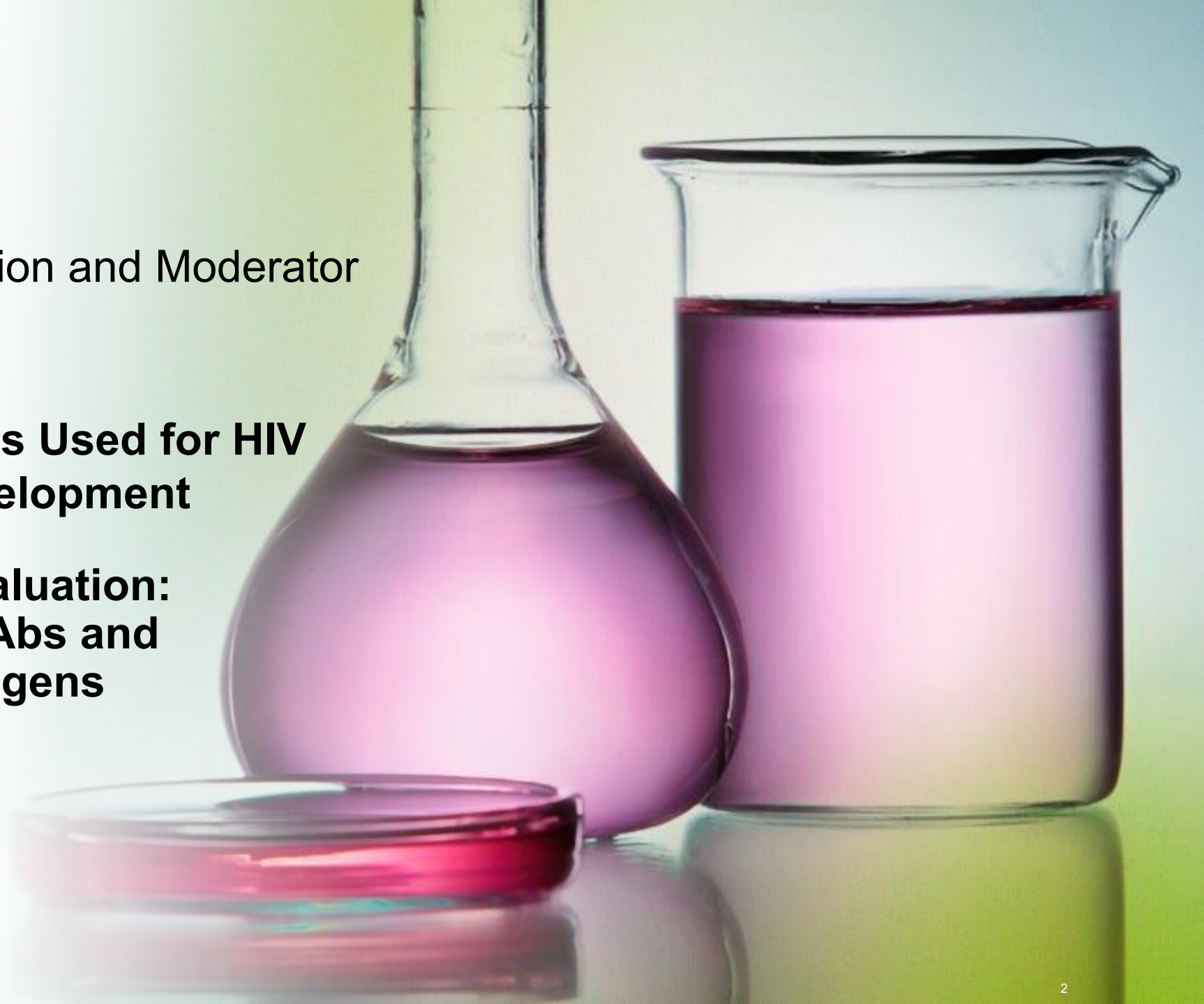
The Raw Material Journey from Research to Results

November 26th, 2025

Scientific excellence for IVD

Hytest

1. Company introduction and Moderator
   Teppo Heimo
2. **Hytest's Strategies Used for HIV Raw Material Development**
3. **Immunoassay Evaluation: Hytest HIV p24 MAbs and Recombinant Antigens**
4. Q&A



Hytest

Who We Are

- 30+ years of dedication to providing top-tier immunological reagents for the IVD industry and research community
- An extensive product portfolio, featuring selected products as standard reference materials
- Active participation in IFCC and ADLM standardization committee work
- Operations compliant with ISO 9001:2015
- Headquarters in Finland and operations in 3 continents with 150+ employees globally
- Products developed based on solid science with 55+ scientific publications
- Close collaborations with KOLs
- Market presence in 55+ countries

Hytest

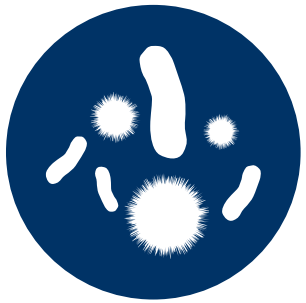
We develop and manufacture world-class antibodies and antigens for the IVD industry, leading to better diagnostics

Hytest

Comprehensive Product Portfolio

Product categories

Monoclonal antibodies
Polyclonal antibodies
Antigens
Sera and Plasma



Clinical Utility

- Screening and monitoring HIV infections in targeted populations to help curb the HIV epidemic
- Early detection and treatment to improve quality of life

HIV monoclonal antibodies for antigen tests

- HIV1/2 p24 (Cat# 3H24)

HIV recombinant antigens for serology tests

- HIV-1, gp120-gp41 N-Trx, rec (Cat# 8H11) ***Available soon!***
- HIV-1, gp41-gp120 N-Fc, rec (Cat# 8H12)
- HIV1 gp41 N-HSA, rec (Cat# 8H13)
- HIV-1, gp120 C-Fc, rec (Cat# 8H16)
- HIV-2, gp36 N-HSA, rec (Cat# 8H24) ***Available soon!***
- HIV-2 gp36 C-TnC, rec (Cat# 8H25) ***Available soon!***

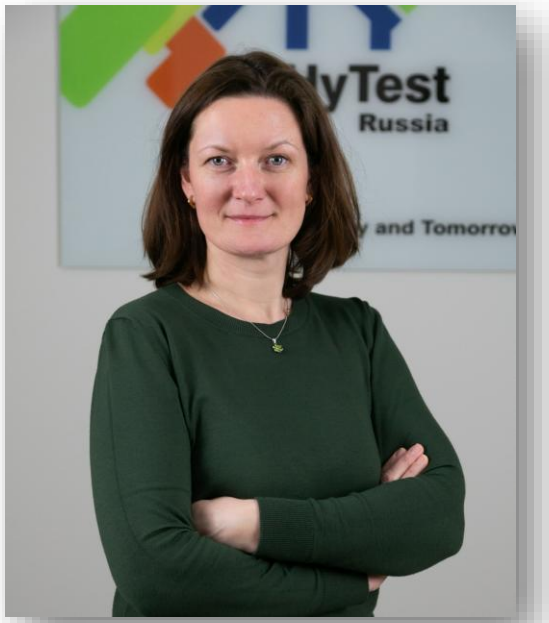
Hytest

Today's Speakers

Natalia Tamm, Ph.D.

Jianwen He, Ph.D.

Natalia Tamm Ph.D.



Leading Researcher and Project Manager, Hytest

M.Sc. and Ph.D. degrees from Moscow State University

Co-author of numerous scientific publications and patents

Joined Hytest in 2003 and has been involved in different research and development projects.

Expertise in a broad range of disease areas, including cardiovascular, infectious disease biomarkers and many others.

Deep knowledge in BNP, proBNP, and NT-proBNP biomarkers

Ongoing research focused on HIV biomarkers

Hytest

Jianwen He Ph.D.

Post-doctorate fellow, University of South California, USA

Doctorate of Medical School of Fudan University (formerly Shanghai Medical University)

Associate Editor of the biblical book“ Immunoassay Handbook” (4th ed. 2013, Publisher Elsevier)

Editor of “Immunoassay: Principle and Application”(2020, Publisher PMPH)

Editor of Chinese-English Bilingual Immunoassay Dictionary (2017, Publisher PMPH)

Published 30 SCI articles

Working and researching on assay principles, technologies & methodologies, and clinical applications of in vitro diagnostics, especially immunodiagnostics, home and abroad

Broad achievements in immunoassay development: biological raw material and reagent design, kinetics and interferences studies, assay and process optimization, clinical application study and troubleshooting

Chief Scientist of IVD BU, Director of IVD Raw Material Business, Mindray

Hytest

Hytest's Strategies Used for HIV Raw Material Development

Natalia Tamm
Ph.D.

Content

- HIV structure and viral genomic diversity
- Development of p24-specific monoclonal antibodies
- Development of HIV-specific recombinant proteins

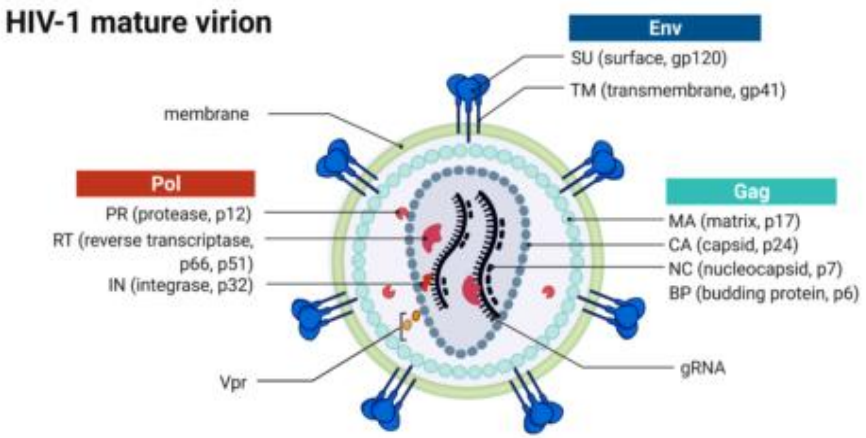
Hytest

Content

- HIV structure and viral genomic diversity
- Development of p24-specific monoclonal antibodies
- Development of HIV-specific recombinant proteins

Hytest

HIV: structure



Li G, De Clercq E. 2016. HIV genome-wide protein associations: a review of 30 years of research. Microbiol Mol Biol Rev

- HIV is a retrovirus causing AIDS (Acquired Immunodeficiency Syndrome).
- Globally, 39.9 million people lived with HIV in 2023
- IVD for detection of HIV infection, not the diagnosis of AIDS

HIV (human immunodeficiency virus)

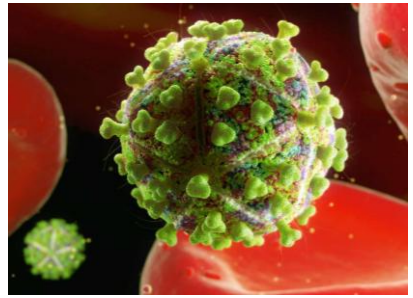
- A member of the genus *lentivirus* in family *Retroviridae* (Retroviruses)
- Sphere with capsid and envelope
- Two copies of positive-sense single-stranded RNA, 9.7kb
- 16 proteins:
  - Structural proteins:
    - Matrix (p17), **capsid (p24)**, nucleocapsid (p7), and p6
  - Viral enzymes:
    - Protease (p12), reverse transcriptase (p66/51), and integrase (p32)
  - Envelope proteins:
    - gp120 and gp41 (HIV-1)/gp36 (HIV-2)
  - Regulatory proteins:
    - Tat and Rev
  - Accessory proteins:
    - Vif, Vpu (HIV-1)/Vpx (HIV-2), Vpr, and Nef

Hytest

HIV: classification, variation and distribution

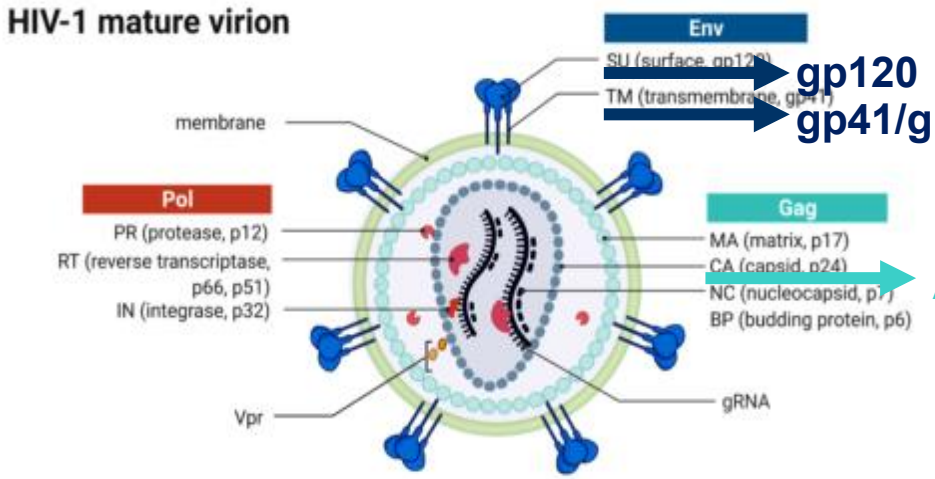
Type | **Group** | **Subtype**

- **HIV-1**
  - ✓ Higher virulence, faster progression
  - **Group P**
    - ✓ Rare
  - **Group O**
    - A
    - B
    - C
    - Cameroon and surrounded countries; Europe, USA
    - ✓ Highly divergent from M in **gag** and **env** regions
  - **Group N**
    - ✓ Very rare
  - **Group M**
    - ✓ Cause of global pandemic
    - **A (A1, A2)** West Africa
    - **B/D** Europe, America, Japan, Thailand, Australia, China
    - **C** South and East Africa, India, Nepal, China
    - D East and Central Africa
    - F (F1, F2) Central Africa, South America, Eastern Europe
    - G Africa, Central Europe
    - H Central Africa
    - J North-Central-West Africa, Caribbean
    - K Congo, Cameroon
    - CRFs and URFs (118 CRFs documented)
- **HIV-2**
  - **Group A-H**
    - Africa, Brazil, Europe, USA
  - ✓ Slower progression, lower transmissibility
  - ✓ Genetically distinct from HIV-1 with ~55% diff in **env** region



Hytest

Specific proteins used for HIV assays development



Antibodies to p24/p26(HIV-2)

Immunoassay requirements

- To design carefully due to genetic diversity
- To detect HIV-1 M subtypes/CRFs, O and HIV-2

Generation	4th Gen
Time	2010
Method	Double-antigen/antibody sandwich
Antigens	Synthetic-peptide or recombinant protein
Antibodies	Monoclonal antibodies to HIV-1 p24 antigen
Analytes	HIV-1 (M and O group) and HIV-2 antibodies, HIV-1 p24 antigen
Feartures	Detection of HIV infection before seroconversion
Problems	Unable to differentiate HIV species
Sereconversion window period	2 weeks

Hytest

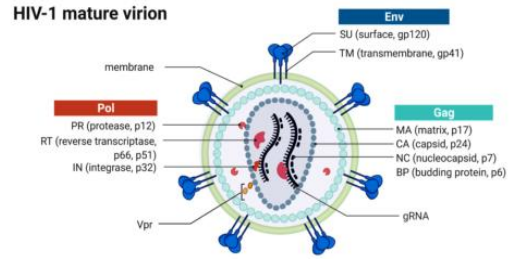
Content

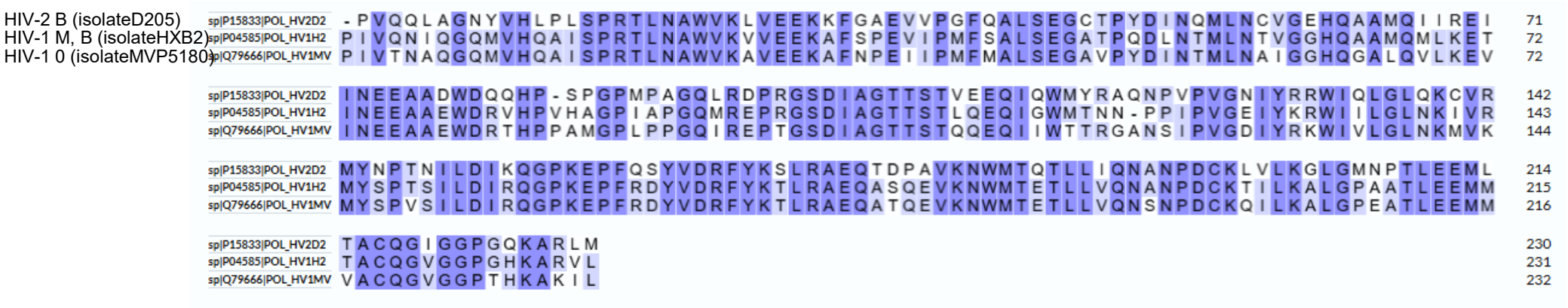
- HIV structure and viral genomic diversity
- Development of p24-specific monoclonal antibodies
- Development of HIV-specific recombinant proteins

Hytest

Hytest HIV-1/2 immunogens: p24 protein

- Structural protein, a component of the HIV capsid
- p24 monomer 230 aar; possibly phosphorylated (hexamer and pentamer)
- Conserved between types and subtypes (see alignment)
- p24 of HIV-1 Group M, HIV-1 Group O, and HIV-2 are related but not identical

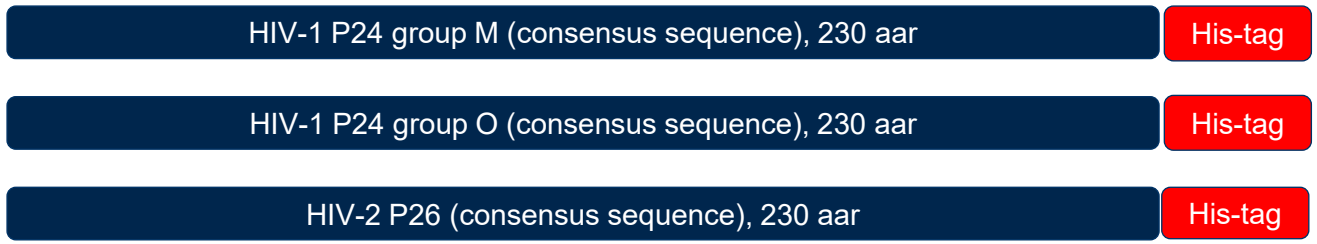




P24 HIV-1 (M-group, subtype B), O-group, p26 HIV-2 alignment. Similarity is highlighted

Hytest

Hytest HIV-1/2 immunogens: p24 proteins design

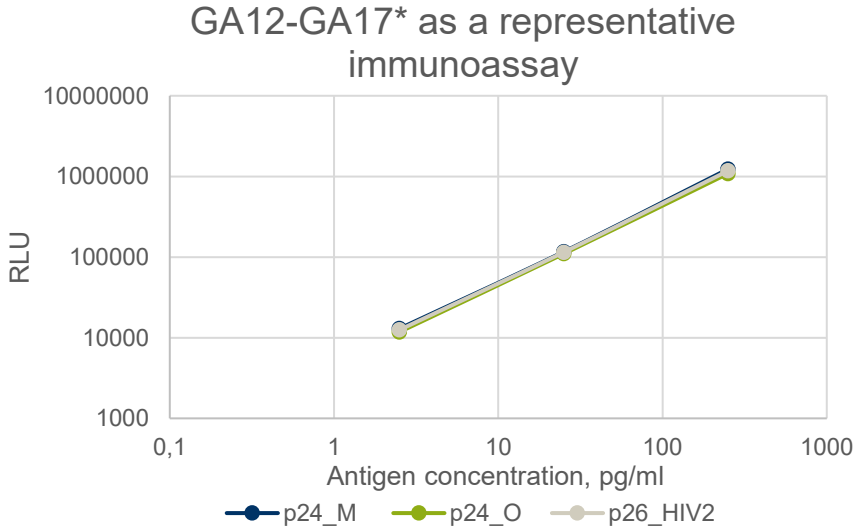


- Expression platform: *E. coli* cells
- Proteins were used for animal immunization and further antibody selection

Hytest immunization strategy

- The immunization strategy included p24 **HIV-1 group M,** p24 HIV-1 **group O and p26 HIV-2** antigens to drive the immune response towards recognition of common epitopes within the p24 antigens of both groups of HIV-1 and also HIV-2.

p24 sandwich immunoassay characteristics



HIV-1 genotypes	S/N ratio
D	17,0
B/G	13,9
A/E	13,9
CRF BF	18,9
A1	15,7
B(214)	16,0
A/G	6,7
B (216)	13,7
H	10,3
C	16,2
CRF G/43_02G	7,9
O	28,7
p26 HIV-2	19,0

The best antibody combinations:

- Recognize p24 HIV-1 gr_M, p24 HIV-1 gr_O, p24 HIV-2 equally effective (the presented antibodies recognize shared epitopes (or conservative fragments) of non-identical p24 proteins
- Recognize the following HIV-1 virus subtypes:
  - A1,B,B,C,D, F1/CRF12_BF/BFrec, G, CRF20_BG, CRF01_AE, CRF02_AG, H, group O (1st WHO International Reference Panel for HIV-1 p24 Antigen NIBSC code: 16/210), and
  - HIV-2 p26 (1st WHO International Reference reagent for HIV-2 p26 Antigen NIBSC code: 16/236)

Hytest

9 MAbs under Cat.# 3H24 (Hytest)

Cat.#	MAb	Origin	Remarks
3H24	GA12	mouse	CLIA, ELISA, *in vitro* monoclonal antibodody
	GA15	mouse	CLIA, ELISA, *in vitro* monoclonal antibodody
	GA17	mouse	CLIA, ELISA, *in vitro* monoclonal antibodody
	GA18	mouse	CLIA, ELISA, *in vitro* monoclonal antibodody
	GA32	rabbit	CLIA, ELISA, recombinant monoclonal antibody
	GA34	rabbit	CLIA, ELISA, recombinant monoclonal antibody
	GA38	rabbit	CLIA, ELISA, recombinant monoclonal antibody
	GA39	rabbit	CLIA, ELISA, recombinant monoclonal antibody
	GA54	rat	CLIA, ELISA, recombinant chimeric antibody

According to the obtained data:

- Specificity to p24 from HIV-1 (group M, group O)/ p26 from HIV-2
- Several MAbs combinations can be suggested for the development of HIV p24 immunoassays
- MAbs could be used on CLIA, ELISA platforms
- HIV infection (HIV-1 or HIV-2) can be detected, including group O, CRF_BC, CRF_AE, and other subtypes using only one antibody pair (2 MAbs) instead of a set of monoclonal antibodies (three, four, a special set), used for HIV diagnostics by several IVD companies

Hytest

Content

- HIV structure and viral genomic diversity
- Development of p24-specific monoclonal antibodies
- Development of HIV-specific recombinant proteins

Hytest

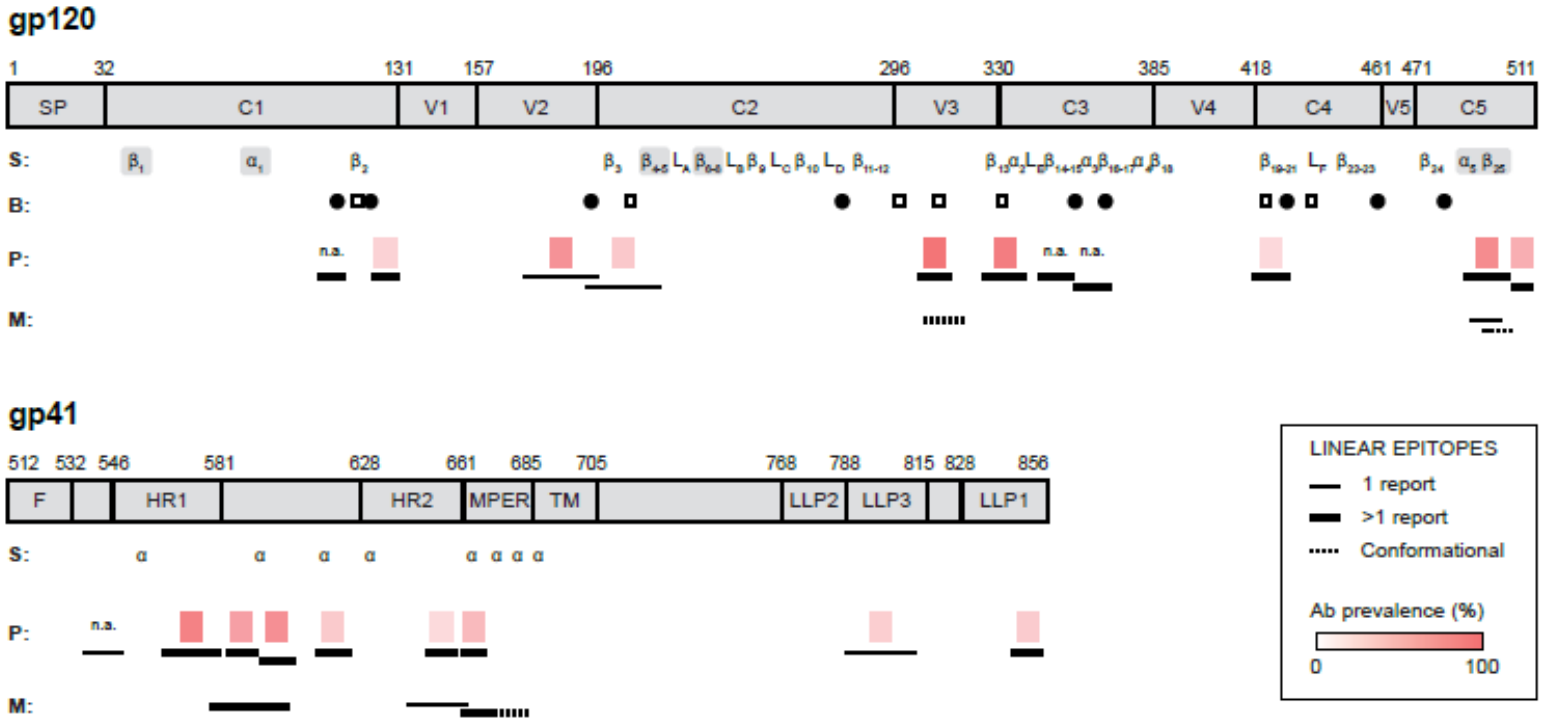
A set of proteins for HIV-1/2 antibody detection

Defining and selecting proteins/epitopes

- High immunogenicity
- Early arising of antibody
- Represent diversity (HIV-1 M/O, subtypes, CRFs; HIV-2)
- Include key immunodominant regions

Hytest

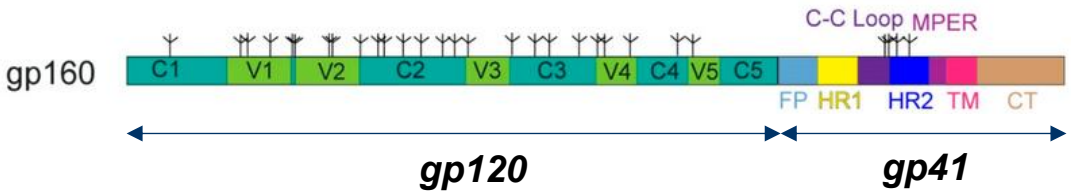
Env proteins represent major molecular targets of HIV-specific antibodies detected in HIV immunoassays



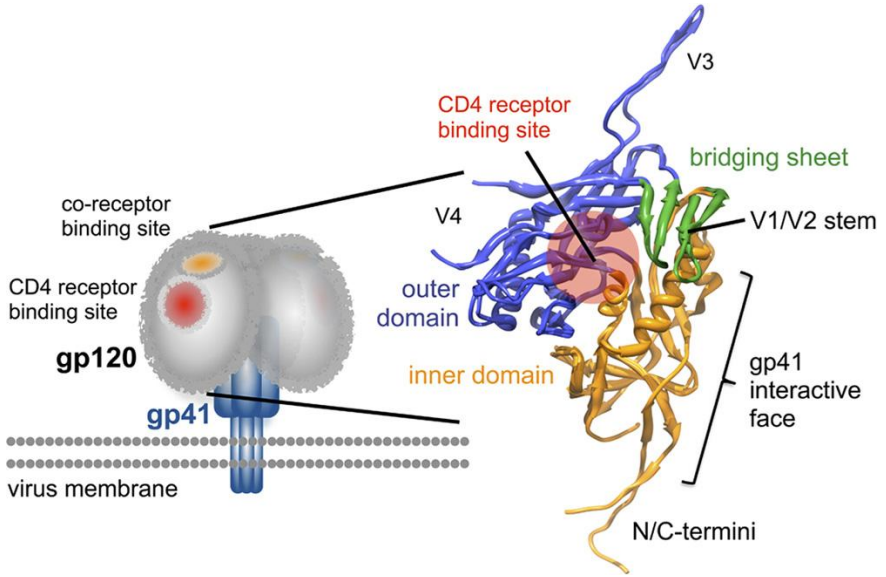
- HIV-1 gp120
- HIV-1 gp41
- HIV-2 gp36

Int Arch Allergy Immunol 2015; 167:223-241

gp120 (env) – one of the proteins used in HIV immunoassays

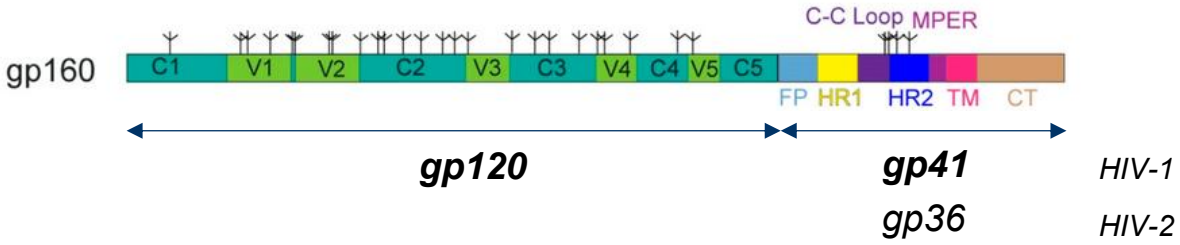


- Envelope glycoprotein
- Trimer
- 481aar HIV-1/503aar HIV-2
- Non-covalently interact with gp41
- V3 and V4 interact with CD4 to trigger viral entry
- Heavily glycosylated (both N- and O-glycans)
- High diversity between gp120 HIV-1/HIV-2



Solution Structure, Conformational Dynamics, and CD4-Induced Activation in Full-Length, Glycosylated, Monomeric HIV gp120; Miklos Guttman et al, Journal of Virology p. 8750–8764 August 2012

gp41 (env) – other protein used in HIV immunoassays



- Transmembrane glycoprotein with several functional regions
- Trimer
- 345aar HIV-1/353aar HIV-2
- Four determined sites of N-glycosylation
- Has several IDRs (immunodominant regions)
- High diversity between gp41 HIV-1 and gp36 HIV-2

```
HIV-1-GP41      AVGIGALFLGFLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVW
HIV-2-GP36      -GVFVLGFLGFLATAGSAMGARSLTLSAQSRTLLAGIVQQQQQLLDVVKRQQEMLRLTVW
                   :   *****.:***:*** *:**:.*:* **:*******::** .:: **.:*:****

HIV-1-GP41      GIKQLQARILAVERYLKDQQLLGIWGCSGKLICTTAVPWNASWSNKSLEQIWNHTTWMEW
HIV-2-GP36      GTKNLQARVTAIEKYLKHQAQLNSWGCAFRQVCHTTVPW-----VNDSLSPDWKNMTWQEW
                * *:****: *:*:***.*  *. ***: : :* *:***     *.**.  *:: ** **

HIV-1-GP41      DREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNITNWLWYIKLFIMIVGG
HIV-2-GP36      EKQVRYLEANISQSLEEAQIQQEKNMYELQKLNSWDILGNWFDLTSWVKYIQYGVHIVVG
                ::::.   : * . :**:* *****  ** :*:.*  * ***::*.*: **:  : ** *

HIV-1-GP41      LVGLRIVFAVLSIVNRVRQGYSPLS-------FQTHLPTPRGPDRPEGIEEEGGERDRDR
HIV-2-GP36      IIALRIAIYVVQLLSRFRKGYRPVFSSPPGYLQQIHIHKDRGQPANEGTEEDVGGDSGYD
                ::.***.: *:.::.*.*:** *:         * *: . **    ** **: * .

HIV-1-GP41      SIRLVNGSLALIWDDLRSLCLFSYHRLRDLLLIVTRIVELLGR---RGWEALKYWWNLLQ
HIV-2-GP36      LWPWPINYVQFLIHLLTRLLIGLYNICRDLLSKNSPTRRLISQSLTAIRDWLRLKAAQLQ
                     . : :: . *  * :  *:  ****   :   .*:.:      : *:     **

HIV-1-GP41      YWSQELKNSAVSLLNATAIAVAEGTDRVIEVVQGACRAIRHIPRRIRQGLERILL
HIV-2-GP36      YGCEWIQEAFQAFARTTRETLAGAWGWLWEAARRIGRGILAVPRRIRQGAELALL
                * .: ::::  :: .:*  ::* . . : *..:   *.*  :******* *  **
```

gp41 HIV-1 and gp36 HIV-2 alignment. Similarity is marked with asterix

Hytest

HIV proteins design; general ideas

Raw materials (proteins)

Global IVD companies (2) presumed design

- Based on available info
- Fusions, recombinant and peptides

Antigen design	Format	Expression platform
HIV-1 gp41_ M	Recombinant protein	*E. coli*
HIV-1 gp41_O	Synthetic peptide	
HIV-1 gp41/gp120	Recombinant protein	*E. coli*
HIV-2 gp36	Recombinant protein, fused	*E. coli*

Hytest design

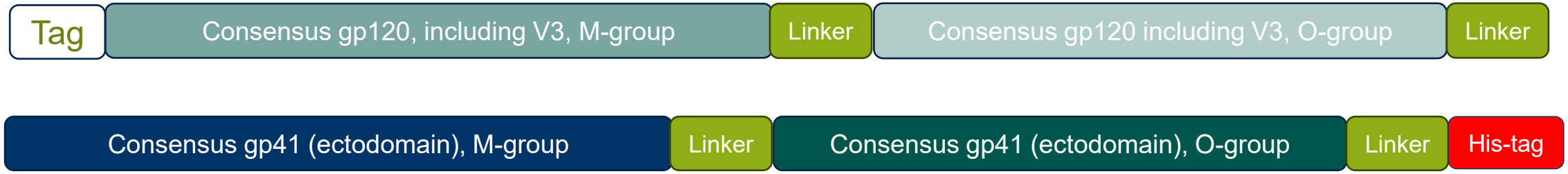
- Maximal diversity
- Consensus sequences
- Different expression platforms for the same protein used for coating and detection
- Sequences based on open-source info

Antigen Design	Format	Expression platform
HIV-1 gp41 _ M	Recombinant protein	Mammalian cells
HIV-2 gp36	Recombinant protein	Mammalian cells
HIV-1 gp120_M,O	Recombinant protein, fused	Mammalian cells
HIV-1 gp120_M,O+gp41_M,O	Recombinant protein, fused	Mammalian cells, *E. coli*

Hytest

HIV-specific proteins development strategies

- The maximal diversity in one recombinant protein

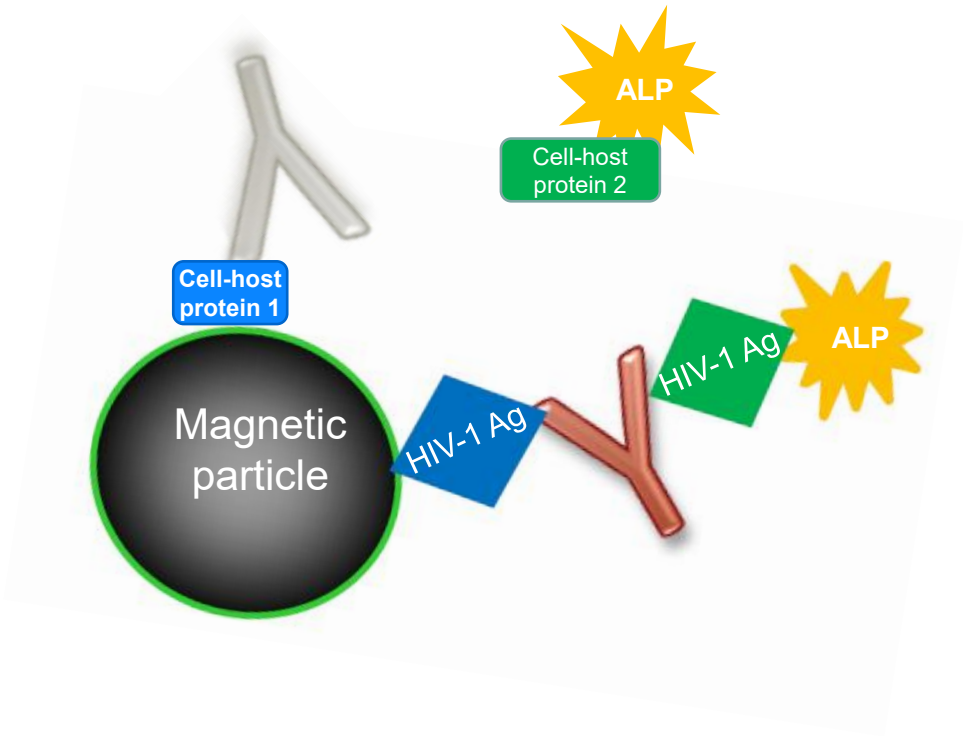


HIV-1 gp120+gp41, fusion protein

Hytest

HIV-specific proteins development strategies

- The same protein used for double antigen sandwich produced in **2 different expression systems**: *E. coli* and mammalian cells to reduce the risk of interference when used in a double antigen sandwich assay



Hytest

HIV-1 gp120+gp41, fusion protein expression

E.*coli* cells

- Recombinant protein gp41/gp120 (fusion) was expressed with protein tag-partner (Thioredoxin)
- No post-translational modifications
- Partly refolded protein
- HIV-1 Trx gp41+gp120 has reactivity with HIV-1 M and O group blood samples (according to the HIV-tests manufactures)
- Acceptable protein stability under different temperature conditions

Mammalian cells

- Recombinant protein gp41/gp120 (fusion) was expressed with protein tag-partner (Fc-fragment of human IgG)
- Post-translational modifications (glycosylation) could be similar to HIV proteins circulating in the blood of infected people
- Soluble protein
- HIV-1 gp41+ gp120 Fc has reactivity with HIV-1 M and O group blood samples (according to the HIV-tests manufactures)
- High protein stability under different temperature conditions

Hytest

HIV-proteins design and expression by mammalian cells

HIV-1 gp41_M ***or*** *HIV-2 gp36*

HIV-1 gp120_M, O

- Recombinant proteins **HIV-1 gp41_M**, **HIV-2 gp36; HIV-1 gp120_M, O** were expressed with protein tag-partners (human serum albumin (HSA) or human cardiac troponin C (TnC) or Fc- fragment of human IgG)
- Post-translational modifications (glycosylation) could be similar to HIV proteins circulating in the blood of infected people
- All proteins are soluble
- **HIV-1 gp41_M**, **HIV-2 gp36; HIV-1 gp120_M, O** have reactivity with blood samples of corresponding group (according to the HIV-tests manufactures)
- High protein stability

Hytest

HIV-specific proteins recommended for HIV Ab assays development

Cat.#	Product name	Purity-% (SDS-PAGE)	Expression system
8H11	HIV-1, gp120-gp41 N-Trx, recombinant	> 80	Bacterial cells
8H12	HIV-1, gp41-gp120 N-Fc, recombinant	> 80	Mammalian cells
8H13	HIV-1, gp41 N-HSA, recombinant	> 80	Mammalian cells
8H16	HIV-1, gp120 C-Fc, recombinant	> 90	Mammalian cells
8H24	HIV-2, gp36 N-HSA, recombinant	> 90	Mammalian cells
8H25	HIV-2 gp36 C-TnC, recombinant	> 80	Mammalian cells

Hytest

Thank you

Natalia Tamm

Immunoassay Evolution: Hytest HIV p24 MAbs and Recombinant Antigens

Jianwen He
Ph.D.

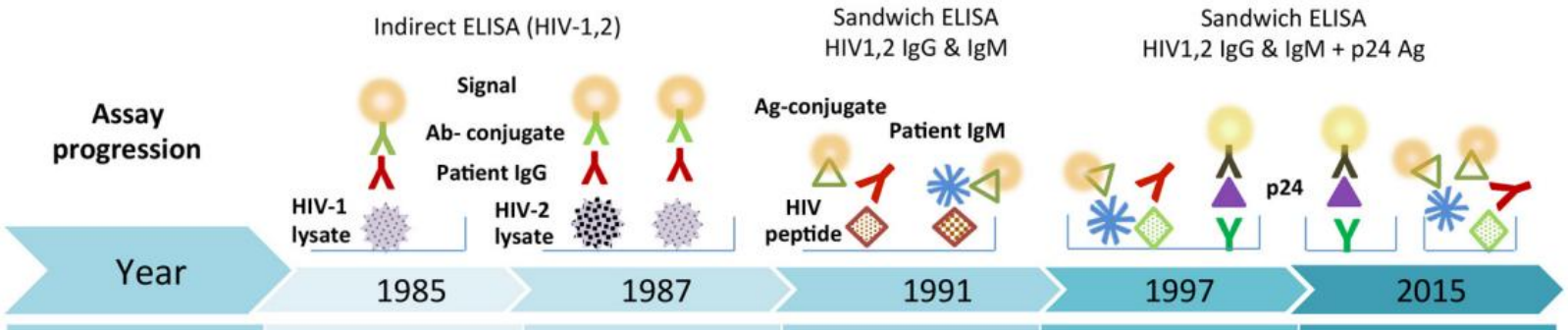
Content

- HIV immunoassay evolution
- Hytest HIV p24 MAbs evaluation
- Hytest HIV recombinant proteins evaluation

Content

- HIV immunoassay evolution
- Hytest HIV p24 MAbs evaluation
- Hytest HIV recombinant proteins evaluation

HIV Immunoassay Evolution



Generation	1st	2nd	3rd	4th	5th
Antigen (Ag) Source	Virus Infected Cell Lysate	Lysate & Recombinant	Recombinant & Synthetic peptides	Recombinant & Synthetic peptides	Recombinant & Synthetic peptides
Specificity	95-98%	>99%	>99.5%	99.5%	99.5%
Sensitivity	99%	>99.5%	>99.5%	>99.8%	100%
Negative Window	8-10 weeks	4-6 weeks	2-3 weeks	2 weeks	2 weeks
Detects Antibody (Ab) and Ag	IgG Anti HIV-1	IgG anti HIV-1 and IgG anti HIV-2	IgG and IgM anti HIV-1, HIV-2 and Group O	IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag	IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag
Results	Single result	Single result	Single result	Single result; does not differentiate Ab from Ag positivity	Separate HIV-1 and HIV 2 Ab and Ag results

Becoming more sensitive and specific

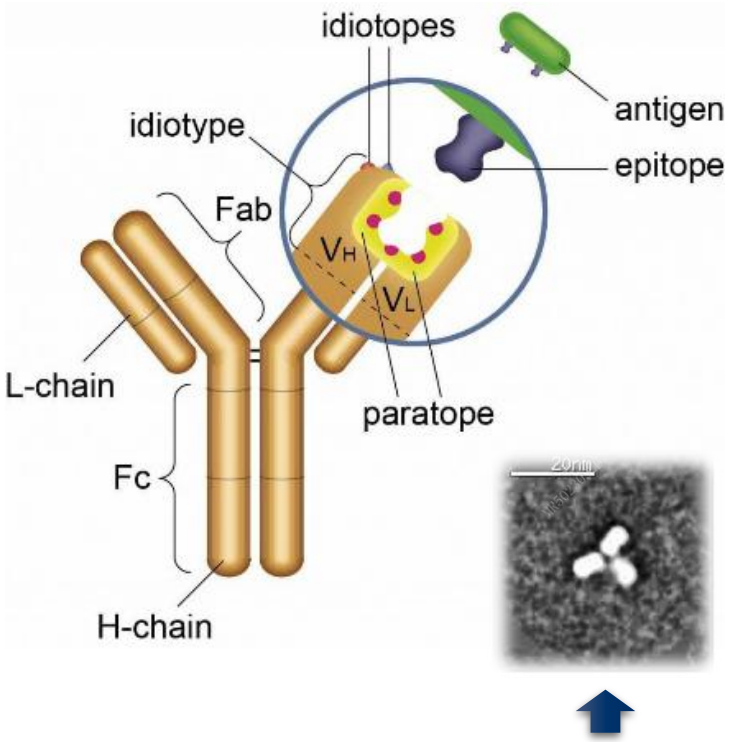
- Lysate to recombinant/Peptides
- IgG to IgG+IgM
- HIV antibodies to antigen (gp24)
- Detection to differentiation (types, groups, subtypes)
- Identifies Infection Stage: quick intervention and short diagnostic algorithm

Alexander TS. Clin Vaccine Immunol. 2016 4;23(4):249-53

Antibody Is at the Heart of Immunoassay

50% IVD product recalls can be traced to raw materials

IgG Structure and Ag Binding Site



IgG in EM: 3 x 7 x 13 nM

Requirements for Antibody

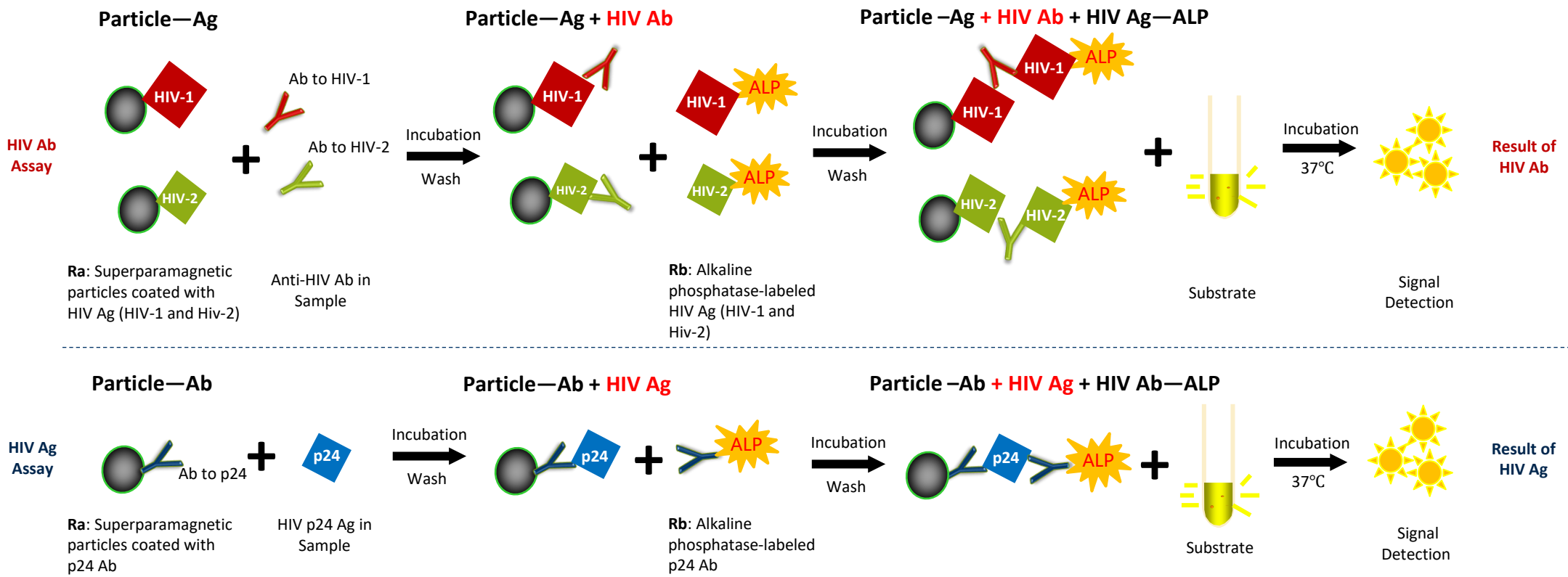
- High affinity
- High purity
- Stability
- Specificity with defined epitopes
- Batch to batch consistency
- Sustainable supply
- In compliance

"A few good antibodies"

Content

- HIV immunoassay evolution
- Hytest HIV p24 MAbs evaluation
- Hytest HIV recombinant proteins evaluation

HIV Immunoassay Principle: Two-step Sandwich



HIV MAb Evaluation
S/N of p24/p26 antigens

Reactivity of Hytest p24 antibody pairs

- MAb pairs demonstrated with high Signal to Noise ratio (S/N) for Hytest HIV-1 M and O group p24 antigens, HIV-2 p26 antigen, and p24 WHO standard (NIBSC code: 90/636).

MAbs evaluated

Cat.#	MAb	Fc
3H24	GA12	Mouse
	GA17	Mouse
	GA32	Rabbit
	GA34	Rabbit
	GA38	Rabbit
	GA39	Rabbit
	GA54	Rat

No.	Capture Mab	Detection Mab	HIV-1 p24 M antigen 10 pg/ml	HIV-1 p24 O antigen 10 pg/ml	HIV-2 p26 antigen 20 pg/ml	p24 WHO standard 2 IU/ml
1	GA12	GA17	10.05	13.52	7.26	8.61
2	GA17	GA54	9.23	14.16	5.89	8.41
3	GA17	GA12	9.31	14.24	7.66	7.70
4	GA32	GA54	9.26	10.22	3.43	7.91
5	GA54	GA17	10.14	13.63	5.78	8.67
6	GA54	GA38	9.30	11.38	5.54	6.74
7	GA12	GA38	7.92	9.48	8.11	7.31
8	GA34	GA54	8.77	11.40	3.48	7.71
9	GA34	GA12	8.12	10.78	7.01	7.33
10	GA39	GA12	8.23	9.58	7.07	7.45
11	GA39	GA54	7.81	9.18	4.11	7.47
12	GA32	GA12	8.24	9.18	6.98	7.29
13	GA38	GA54	7.90	11.00	3.67	6.70
14	GA17	GA34	8.09	9.97	4.89	6.71
15	GA54	GA34	8.35	9.64	4.57	6.59
16	GA17	GA38	8.18	10.59	5.23	6.55
17	GA34	GA38	8.70	9.70	7.91	5.80
18	GA12	GA34	7.29	8.98	6.23	6.96
19	GA34	GA39	7.92	7.62	7.50	6.12
20	GA38	GA12	7.24	8.98	5.86	6.10
21	GA32	GA34	8.45	6.45	5.81	6.06
22	GA38	GA34	8.58	9.01	7.97	5.73
23	GA39	GA34	7.56	6.46	5.86	6.17

HIV MAb Evaluation
Limit of Detection (LoD)

- Hytest MAb pairs showed LoD of WHO international standard (NIBSC code: 90/636) about 0.5~0.65 IU/ml, which meets the requirement of COMMISSION IMPLEMENTING REGULATION (EU) 2022/1107 (≤ 2 IU/ml).
- Most of the MAb pairs showed a greater detection capacity for HIV-1 p24 and p26 antigens than results generated in compared groups.

Number of Pair	Capture MAb	Detection MAb	LOD of WHO international standard p24 antigen, IU/ml	LOD HT HIV-1 p24_M, pg/ml	LOD HT HIV-1 p24_O, pg/ml	LOD HT HIV-2 p26, pg/ml
1	GA17	GA54	0.64	4	1.5	15
2	GA17	GA12	0.64	2	2	8
3	GA17	GA38	0.55	1.5	1.5	8
4	GA34	GA54	0.5	2	1.5	20
5	GA38	GA54	0.55	4	1.5	20
6	GA17	GA34	0.64	4	2	15
7	GA39	GA54	0.64	4	4	25
8	GA17	GA32	0.64	2	2	8
9	GA32	GA54	0.55	4	4	30
10	GA17	GA39	0.64	2	2	8
11	GA34	GA39	0.55	2	4	5
12	GA34	GA38	0.5	1	1	3
13	GA38	GA12	0.55	2	1.5	8
14	GA12	GA34	0.55	2	2	8
15	GA12	GA32	0.64	4	4	4
16	GA12	GA39	0.64	4	4	4
17	GA34	GA32	0.55	2	4	8
	IVD Combo1		1.3	5	10	20
	IVD Combo2		1.0	5	4	Not detected

HIV MAb Evaluation
Specificity

- Evaluated with a variety of sample groups, Hytest MAb pairs showed that specificity meets requirement of COMMISSION IMPLEMENTING REGULATION (EU) 2022/1107 (specificity >99.5%).

Capture MAb / Detection MAb			GA17 GA12	GA17 GA54	GA17 GA38	GA17 GA34	GA17 GA32	GA17 GA12+GA38
Sample Category		**Sample Number**	**Number of false reactive (%)**					
Collected Samples	Study 1	1000	1 (99.90%)	/	0 (100%)	1 (99.90%)	0 (100%)	1 (99.90%)
	Study 2	1014	0 (100%)	/				
Clinical Samples	Study 1	1228	0 (100%)	0 (100%)				
	Study 2	941	0 (100%)	0 (100%)		/		
	Study 3	1251	2 (99.84%)	2 (99.84%)				
Blood Donor Samples		989	0 (100%)	0 (100%)				
Total	**Samples number**	**6423**	**6423**	**4031**	**1000**	**1000**	**1000**	**1000**
	False Reactive	**/**	**3 (99.95%)**	**2 (99.95%)**	**0 (100%)**	**1 (99.90%)**	**0 (100%)**	**1 (99.90%)**

HIV MAb Evaluation
Reference Panel 1

- Hytest MAb pairs showed higher sensitivity than compared groups in detection of p24 and p26 antigen, respectively.

Assay			Hytest Pair GA17 + GA12	Hytest Pair GA17 + GA54	IVD Combo1	IVD combo2
Sample	Genotype	Conc.	COI	COI	COI	COI
p24 WHO Reference Panel 16/210	A1	1 IU/ml	3.35	2.92	0.881	2.29
	B 16/214	1 IU/ml	4.82	4.16	1.55	2.35
	B 16/216	1 IU/ml	3.62	3.17	1.64	1.26
	C	1 IU/ml	3.58	3.26	1.23	1.96
	D	1 IU/ml	4.16	3.57	1.1	2.51
	F1	1 IU/ml	3.39	3.04	1.83	1.26
	G	1 IU/ml	2.55	2.12	1.4	2.21
	BG	1 IU/ml	3.68	3.28	1.57	1.43
	AE	1 IU/ml	3.26	2.68	1.31	2.5
	AG	1 IU/ml	3.6	2.93	1.62	1.86
	H	1 IU/ml	4.49	3.92	1.26	2.12
	Group O	1 IU/ml	6.48	5.41	2.06	3.56
HyTest Antigen	p24_M	2pg/ml	1.22	0.9	0.678	0.46
	p24_O	2pg/ml	1.85	1.38	0.54	0.96
	HIV-2 p26	20pg/ml	3.02	1.54	5.95	0.38
	HIV-2 p26	10pg/ml	1.8	0.91	0.653	0.11

HIV MAb Evaluation
Reference Panel 2

- Hytest MAb pairs showed higher sensitivity than compared groups in detection of p24 and p26 antigen, respectively.

Assay			Hytest Pair GA17 + GA12	Hytest Pair GA17 + GA54	IVD Combo1	IVD combo2
Sample	Genotype	Conc.	COI	COI	COI	COI
p24/p26 Reference Panel From A Third Party	B	5pg/ml	1.40	1.10	1.56	1.29
	CRF-07-BC	5pg/ml	1.38	1.01	1.16	0.11
	CRF-07-BC	5pg/ml	1.46	1.33	1.16	0.85
	CRF-01-AE	5pg/ml	1.61	1.13	1.42	1.82
	CRF-01-AE	5pg/ml	1.20	1.09	2	1.71
	CRF-08-BC	5pg/ml	1.70	1.23	1.16	1.38
	CRF-08-BC	10pg/ml	1.97	1.46	1.49	0.23
	CRF-55-01B	5pg/ml	1.54	1.41	1.54	1.88
	CRF-55-01B	5pg/ml	2.13	1.58	1.93	2.59
	A1	15pg/ml	1.49	1.17	2.01	1.21
	B	15pg/ml	3.75	1.47	1.33	0.97
	B	10pg/ml	1.82	1.53	2.04	0.14
	C	5pg/ml	1.56	1.35	1.12	0.37
	D	10pg/ml	1.64	1.42	0.989	0.21
	F1/CRF12-BF/BFrec	5pg/ml	1.09	0.97	1.29	0.1
	G	30pg/ml	1.57	1.54	1.91	0.4
	CRF20-BG	5pg/ml	1.22	1.10	1.34	0.14
	CRF01-AE	5pg/ml	1.43	1.19	1.11	1.22
	CRF02-AG	5pg/ml	1.40	1.21	1.41	0.68
	H	5pg/ml	1.50	1.46	1.06	0.36
	HIV-1 O	10pg/ml	1.79	1.28	0.394	1.88
	HIV-2 A	250pg/ml	2.77	1.11	4.86	0.25

Content

- HIV immunoassay evolution
- Hytest HIV p24 MAbs evaluation
- Hytest HIV recombinant proteins evaluation

HIV Antigen Evaluation- Sensitivity

- 11 undiluted positive HIV-1 samples were evaluated, and the Hytest HIV-1 antigen pairs demonstrated sensitivity of 100%. 11 different diluted HIV-2 samples were tested, and the Hytest HIV-2 antigen pairs demonstrated sensitivity of 100%.

Recombinant Antigens Evaluated

No.	Cat. #	Antigen	Expression System
1	8H11	HIV-1, gp120-gp41 N-Trx, rec	*E. coli*
2	8H12	HIV-1, gp41-gp120 N-Fc, rec	Mammalian cells
3	8H13	HIV-1, gp41 N-HSA, rec	Mammalian cells
4	8H24	HIV-2, gp36 N-HSA, rec	Mammalian cells
5	8H25	HIV-2, gp36 C-TnC, rec	Mammalian cells

HIV-1 Positive Samples

Capture Antigen / Detection Antigen	8H11 / 8H12	8H11 / 8H13	IVD Combo1	IVD Combo2
No.1	1.00	3.23	59.228	3.53
No.2	1.49	2.34	6.621	3.69
No.3	4.13	5.34	18.691	9.88
No.4	4.77	51.76	9.308	8.13
No.5	18.07	46.44	25.498	49.74
No.6	23.16	47.99	24.068	102.2
No.7	23.86	61.58	38.319	138.2
No.8	39.49	77.6	45.984	294.4
No.9	52.72	81.08	29.218	161.9
No.10	56.84	107.28	93.722	217
No.11	188.54	235.45	93.499	190.4

HIV-2 Positive Samples

Capture Antigen / Detection Antigen	8H24 / 8H24	8H24 / 8H25	8H25 / 8H24	IVD Combo1
No.1	1.74	1.90	1.86	1.76
No.2	3.62	2.41	4.45	7.51
No.3	12.49	8.53	20.41	15.92
No.4	14.88	12	26.5	22.02
No.5	4.12	2.93	9.91	1.48
No.6	19.72	14.68	49.99	26.83
No.7	17.5	14.89	44.1	34.67
No.8	10.42	10.72	20.45	5.58
No.9	16.81	14.47	39.01	32.13
No.10	24.64	20.92	66.67	45.01
No.11	20.46	18.4	54.66	37.18

HIV Antigen Evaluation- HIV-1 Specificity

- Evaluated using various types of interference samples and clinical specimens, 2 Hytest HIV-1 antigen pairs demonstrated high specificity, satisfying the required performance criteria of COMMISSION IMPLEMENTING REGULATION (EU) 2022/1107 (specificity >99.5%).

Particle Antigen			8H11	8H11
Conjugate Antigen			8H12	8H13
Type of Interference		Sample Number	False Reactive	
Interference Sample		78	1	3
ANA Positive Sample		88	0	0
RF Sample		139	0	1
Total	**Sample Number**	**305**	**305**	**305**
	False reactive	**/**	**1**	**4**
Sample Category		**Sample Number**	**False Reactive**	
Collected Samples	Study 1	856	3 (99.65%)	3 (99.65%)
	Study 2	1014	/	3 (99.70%)
Clinical Samples	Study 1	850	0 (100%)	0 (100%)
	Study 2	941	/	1 (99.89%)
	Study 3	1251	1 (99.92%)	1 (99.92%)
Blood Donor Samples		989	/	2 (99.80%)
Total	**Sample Number**	**5901**	**2957**	**5901**
	False reactive	**/**	**4 (99.86%)**	**10 (99.83%)**

HIV Antigen Evaluation- HIV-2 Specificity

- Evaluated with different types of interference samples and clinical samples, 4 Hytest HIV-2 antigen pairs had high specificity, which met the requirement (specificity >99.5%).

Particle Antigen			8H24	8H24	8H25	8H25
Conjugate Antigen			8H24	8H25	8H24	8H25
Type of Interference		**Number**			**False Reactive**	
Interference Sample		78	0	0	0	0
ANA Positive Sample		88	/	0	/	/
RF Sample		102	/	0	/	/
Total	**Sample Number**	**268**	**78**	**268**	**78**	**78**
	False Reactive	**/**	**0**	**0**	**0**	**0**
Sample Category		**Number**			**False Reactive**	
Collected Samples	Study 1	1638	0 (100%)	0 (100%)	0 (100%)	1 (99.87%)
	Study 2	1014	0 (100%)	0 (100%)	/	/
Clinical Samples	Study 1	850	/	0 (100%)	/	/
	Study 2	941	/	0 (100%)	/	/
	Study 3	1251	/	0 (100%)	/	/
Blood Donor Samples		989	/	0 (100%)	/	/
Total	**Sample Number**	**6683**	**2652**	**6683**	**1638**	**750**
	False Reactive	**/**	**0 (100%)**	**0 (100%)**	**0 (100%)**	**1 (99.87%)**

HIV Ab/Ag Combo Evaluation-Genotype

- Hytest HIV Ab/Ag combo assays were able to detect all different genotypes similar to compared groups.

Assay Pairs No.	HIV Subtype	HIV-1 Viral Load*	HIV Combo1 HIV-1: 8H11+8H13 HIV-2: 8H24+8H25 p24: GA17+GA12 COI	HIV Combo2 HIV-1: 8H11+8H12 HIV-2: 8H24+8H25 p24: GA17+GA54 COI	IVD Combo1 COI	IVD Combo2 COI
1	A	262,186	13.73	18.01	25.5	16.17
2	B	46	145.41	246.52	821	1038.44
3	B	1,361,613	349.45	673.57	1625	566.84
4	B	761,620	432.19	781.81	274	940.22
5	C	> 10,000,000	6.20	7.48	5.66	3.33
6	C / CRF_BC	1,427,474	25.84	23.03	66.7	69.84
7	C / CRF_BC	> 10,000,000	11.58	10.38	7.09	6.7
8	C / CRF_BC	> 10,000,000	53.14	57.85	36.8	17.19
9	C / CRF08_BC	98,128	4.17	4.61	44.1	30.92
10	C / CRF08_BC	1,076	34.85	37.51	188	53.09
11	C / CRF08_BC	90,675	268.82	429.63	280	452.14
12	C / CRF31_BC	10,594	232.53	451.57	2788	1157.45
13	CRF_BG	2,711	220.97	396.42	137	620.13
14	CRF01_AE	12,688	100.72	162.14	251	257.72
15	CRF02_AG	> 10,000,000	13.27	14.14	10.3	6.44
16	CRF03_AB	163	44.32	47.49	43.1	9.04
17	CRF22_01A1	548,193	170.99	304.56	41.4	189.41
18	D	243,267	4.01	4.53	4.16	**0.55**
19	F2	28,294	348.48	680.02	404	279.91
20	G	9,629	387.05	711.00	1055	774.07
21	/	> 10,000,000	6.95	9.32	7.37	4.63
22	/	> 10,000,000	2.42	2.30	1.96	1.95

*All tested samples are diluted 100 times

HIV Ab/Ag Combo Evaluation-Seroconversion Panel

- Hytest HIV Ab/Ag combo assays demonstrated similar sensitivity as compared group.

Assay / Pairs			HIV Combo1 HIV-1: 8H11+8H13 HIV-2: 8H24+8H25 p24: GA17+GA12	HIV Combo2 HIV-1: 8H11+8H12 HIV-2: 8H24+8H25 p24: GA17+GA54	IVD Combo1
No.	Seroconversion Panel	Sample Size	Number of HIV reactive samples		
1	HIV9021	17	4	4	4
2	HIV9018	9	3	3	3
3	HIV9030	16	3	3	3
4	HIV9011	11	2	2	2
5	HIV9028	7	2	2	2
6	HIV 9012	8	3	**4**	3
7	HIV 9077	23	12	12	12
8	SCP-HIV-002	20	11	**12**	11
9	SCP-HIV-003	7	4	4	4
10	SCP-HIV-004	7	3	3	3
11	SCP-HIV-005	25	21	21	21
12	SCP-HIV-007	9	4	4	4
13	0600-0240	5	2	2	2
14	0600-0245	7	2	2	2
15	0600-0248	10	3	3	3
16	0600-0251	10	**3**	4	4
17	0600-0256	4	2	2	2
18	0600-0258	4	2	2	2
19	0600-0261	4	**3**	**3**	2
20	0600-0270	4	**3**	**3**	2
21	0600-0272	6	3	3	3
22	0600-0243	9	2	2	2
Seroconversion Panel Tested			22	22	22
Total Tested Samples			222	222	222
HIV Reactive Samples			**97**	**100**	**96**

HIV Ab/Ag Combo Evaluation-Specificity

- A total of 4272 different types of interference samples and samples from different populations were evaluated with HIV Ab/Ag combo assays.
- Hytest HIV Ab/Ag combo assays showed high specificity (>99.9%).

HIV Ab/Ag Combo Evaluation

Assay	HIV Combo1	HIV Combo2
HIV-1 pair	8H11 + 8H13	8H11 + 8H12
HIV-2 pair	8H24 + 8H25	8H24 + 8H25
p24 pair	GA17 + GA12	GA17 + GA54
Sample number	4272	4272
Number of false reactive	**3**	**4**
Specificity	**99.93%**	**99.91%**

Sources of Evaluated Samples

Assay / Sample Category	Number Tested	HIV Combo1 Number of False Positive	HIV Combo2 Number of False Positive
Hospital 1	1690	2	2
Hospital 2	40	0	0
Hospital 3	739	0	2
Hospital 4	213	0	0
Triglyceride-rich Samples	189	0	0
Hemolyzed Samples	189	0	0
RF Samples	69	0	0
ANA Positive Samples	299	1	0
IgM-rich Samples	2	0	0
Patients with Diabetes	95	0	0
Patients with Uremia	172	0	0
Patients with Dialysis	8	0	0
Old People	124	0	0
Pregnant women	75	0	0
Newborn	100	0	0
Children	84	0	0
Cardiovascular Diseases	184	0	0
Total	**4272**	**3**	**4**

Conclusions

- The evaluated HIV p24 monoclonal antibodies using Mindray CLIA System exhibit high sensitivity and specificity in the detection of both p24 and p26 antigens.

- The assessed HIV recombinant antigens using Mindray CLIA System demonstrate high sensitivity and specificity.

Hytest

mindray 迈瑞
生命科技如此亲近

Thank you

Jianwen He

Hytest

Q&A

Q&A

Q: Can you explain your quality control process that you are using for your (Hytest's) products?

A: Hytest's quality control process can be described like this:

-We use raw materials/chemical reagents only from suppliers who are approved on our list of acceptable suppliers (based on quality, reliability etc) and we follow performance of our suppliers continuously

-In-coming inspection is performed on the materials (this is in many case a document review to confirm acceptable specifications etc.)

-Only raw material from product specific list of reagents can be used in manufacturing and QC, the reagents on the list are carefully selected and qualified by R&D before product launch

-All product lots are QC-tested to confirm that lots correspond to internal specifications and pass predetermined QC acceptance criteria. Hytest uses a broad variety of QC tests for produced proteins, such as appearance control, purity determined by means of SDS-PAGE or capillary electrophoresis, proteins' immunoreactivity determined by means of sandwich immunoassays which use protein-specific monoclonal or polyclonal antibodies.

Hytest

Q&A

Q: Why develop 5th-gen assays, when 4th-gen is "good enough"?

A: 4th-generation assays are indeed effective and widely utilized; however, they yield only qualitative "positive/negative" results for antibodies and do not provide information regarding the stage of infection. In contrast, 5th-generation assays offer significant additional advantages. First, they enable differentiation among HIV-1 p24 antigen, HIV-1 antibodies, and HIV-2 antibodies. This enhanced analytical resolution facilitates earlier detection of acute infections and reduces the necessity for confirmatory testing—potentially lowering associated testing costs by up to 40%. In markets such as those in Europe and the United States, where molecular diagnostic testing is particularly costly, this capability represents a compelling value proposition.

Hytest

Q&A

Q: How critical is recombinant viral protein stability in 4th/5th-gen reagents?

A: Recombinant viral protein stability is of critical importance for both fourth- and fifth-generation HIV assays.

From the raw material supplier's perspective, the stability of HIV-specific proteins represents a key determinant in the performance of HIV-specific reagents. The use of mammalian expression systems enables the production of proteins with high intrinsic stability during manufacturing, storage, and handling. At Hytest, we systematically optimize protein expression, purification, and storage conditions to maximize protein stability. Furthermore, proper posttranslational modifications and the strategic inclusion of protein tags contribute significantly to the enhanced stability of HIV proteins produced in mammalian cells.

From the assay developer's standpoint, even highly stable recombinant proteins may remain susceptible to aggregation and, in some cases, degradation after incorporation into the assay format—whether immobilized as coated antigens or used as conjugated detection reagents. Therefore, careful selection of raw materials is essential, and stability must be actively maintained through thoughtful reagent design and formulation. This involves optimizing buffer composition, detergents, stabilizers, and overall process parameters to ensure consistent assay performance, particularly given the high sensitivity required in HIV testing.

Reliable performance in fourth- and fifth-generation HIV assays is thus achieved through a combination of robust upstream protein production and meticulous downstream assay formulation.

Q&A

Q: Can synthetic peptides replace recombinant antigens?

A: Synthetic peptides can't fully replace recombinant proteins. HIV proteins have several epitopes or antigenic determinants for antibody production on their surface. Epitopes can be either continuous or linear, in which several amino acids, usually from six to eight, are present in the sequence; or discontinuous, in which the amino acids that form the epitope are brought together by the three-dimensional folding of the protein. Even though an epitope constitutes only a relatively few amino acids, its reactivity with the antibody is influenced by amino acids not included in the epitope, or by the presence of post translational modifications, which may also be located outside the epitope.

Moreover, it's easier to take into account the genetic diversity while using a recombinant protein, not the single peptide. More epitopes you have in your protein sequence, the more likely you will be able to determine the presence of specific antibodies.

In some cases, to ensure higher sensitivity in the context of recognizing a wide variety of strains, a specific peptide can be added to the test system, but it's not possible to replace the test system with peptides completely. However, as we know there are approaches in the market, which combine the recombinant proteins with the gp41-specific peptides.

Hytest

Q&A

Q: Is it necessary and if so, how often and in what way should IVD companies (test system manufacturers) evaluate/verify the current HIV assay for its ability to detect newly emerging HIV strains?

A: HIV is a highly genetically variable virus and circulating recombinant forms (CRFs) continue to emerge and spread globally. In vitro diagnostic (IVD) manufacturers should routinely evaluate their HIV immunoassay reagents for the ability to detect emerging HIV strains and CRFs based on a risk-based approach. While there is no universally defined interval for such evaluations, the following strategies are recommended:

1. Maintain an updated inventory of newly identified HIV-1 group M subtypes, novel CRFs, and mutations that may impact antigen–antibody binding. Risk assessments should be periodically revised based on new findings to enable early identification of potential assay vulnerabilities.

2. Continuously monitor field complaints, proficiency testing outcomes, regulatory alerts, and scientific literature. If data trends indicate reduced assay sensitivity in specific geographic regions or sample types, this should prompt immediate reassessment.

3. Conduct formal re-validation when high-risk viral variants are detected. Such evaluations should employ test panels containing recent clinical isolates and newly characterized CRFs to verify that the assay continues to meet its claimed sensitivity and specificity performance.

4. Perform routine lot-to-lot verification testing using HIV reference panels as required by regulatory authorities to ensure manufacturing consistency. Any modifications to antigens, reagents, or production processes should also trigger targeted re-evaluation for strain coverage.

Implementing these measures provides a robust framework for ensuring reliable HIV detection across evolving viral strains and emerging CRFs, thereby supporting long-term assay performance and public health surveillance.

Q&A

Q: Do you have experience in the use of anti-p24 monoclonal antibodies in lateral flow assays?

A: At this stage, we have not conducted internal testing specifically on the lateral flow (LF) platform. However, several customers are currently evaluating our anti-p24 antibodies in their own LF assay development, and these projects are ongoing. While we cannot yet share finalized data, the initial feedback from these evaluations has been positive. Because lateral flow systems can vary significantly in design, materials, and assay configuration, performance is often highly platform-dependent. For this reason, we strongly encourage customers to test the antibodies directly in their own LF setup to obtain the most accurate, application-specific results.

Q&A

Q: Thank you for the excellent talk! Can you speak to stability testing done for the recombinant HIV proteins?

A: During the product development phase, we conduct both accelerated stability studies and freeze–thaw stability evaluations on our recombinant HIV proteins. When studying stability, the change in protein immunoreactivity is assessed using a sandwich immunofluorescence assay using polyclonal antibodies specific to the protein. Protein resistance to freeze/thaw and to the storage under different temperature conditions is also assessed using SDS-electrophoresis. These tests allow us to confirm that the proteins remain stable under various stress conditions.

Thank you!

HYTEST LTD

Intelligate 1, 6th floor,
Joukahaisenkatu 6 • FI-20520 Turku FINLAND

Tel. +358 2 512 0900
E-mail: hytest@hytest.fi

hytest.fi