Title: p24 revisited: A landscape review of antigen detection for early HIV diagnosis

Supplemental Digital Information

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Supplementary Table 1

Study	Assays used for early detection	Detection time relative to reference test	Reference test
Hashida 1996 [1]	Immune complex transfer EIA (for Ab and Ag)	Ag - 7-21 days before	ELISA, agglutination test (for Ab), and Western blot
Sickinger 2004 [2]	Combined-format HIV antigen-antibody (AxSYM HIV Ag/Ab Combo)	6.15 days before	3rd generation Ab tests
Weber 2002 [3]	Enzyme immunoassay (Cobas Core HIV Combi EIA)	3.6 to 5.7 days before	3rd generation Ab tests
		2.75 days after	RT-PCR
Weber 2006 [4]	Fifteen 4th generation assays (VIDAS DUO Ultra to Vironostika HIV uniform II Ag: Ab)	2.4-6.8 days after	PCR
	Two 3rd generation assays (Genscreen HIV 1/2, v2 to Ortho HIV-1/HIV-2 Ab capture)	6.15-10.25 days after	PCR

Supplementary Table 1 Summary of the timing of detection of different assays to detect HIV in comparison to reference and p24 assays. Ag – antigen, Ab – antibody.

Supplementary Table 2

Name	Manufacturer	Applicability	Sensitivity (%)	Specificity (%)	References
AxSYM Combo	Abbott Laboratories	Laboratory	99.6-100	98.0-100	[2,5-11]
Architect HIV	Abbott Laboratories	Laboratory	99.9-100	99.5-100	[6-8,12-14]
VIDAS HIV DUO Ultra/Quick	bioMerieux	Laboratory	95.3-100	98.1-100	[3,5,10,11,15,16]
LG HIV Ag–Ab ELISA PLUS	LG	Laboratory	100	99.9	[17]
Elecsys Combi	Roche	Laboratory	100	99.8-99.9	[7,8,12,18]
Elecsys HIV Combi PT	Roche	Laboratory	100	99.8-100	[6,8,12,18]
Enzygnost HIV Integral	Siemens	Laboratory	100	99.2-100	[3,5,16,19]
GS HIV Combo Ag/Ab EIA	Siemens	Laboratory	100	96.7-100	[9,20,21]
Genscreen Ultra HIV Ag/Ab	Bio-Rad	Laboratory	99.7-100	98.1-100	[3,11,16,22]
Vironostika HIV-1 p24 antigen assay	bioMerieux	Laboratory	91.1-100	96.7-100	[11,12,15,16,19]
BioPlex 2200 HIV Ag-Ab assay	Bio-Rad	Laboratory	100	99.56	[23]
Alere Determine HIV-1/2 Ag/Ab	Alere	PoC/Lab	Acute infection (Ag+/Ab-; Ag+/Ab+)		[24-36]
			Ag+: 0-82.1 Ab+: 0-66.7	0-100	
			Established infection (Ag+/Ab+; Ag-/Ab+)		
			88.2-99.8	89.5-100	

Supplementary Table 2: Commercial assays for p24, with a summary of sensitivity and specificity derived from published studies. Of the assays listed, only the BioPlex 2200 and Alere Determine give separate

readouts for antigen and antibody results. Only studies from independent published trials are included. See Supplementary Table 3 for a breakdown of results from each study (including subsets that included samples from acute infection).

Supplementary Table 3

Reference	Sample type(s)	Index Test	Reference method	Sensitivity[§] (%)	Specificity[§] (%)	N (samples)
Chandwani 1993 [37]	Infants <14 days, plasma, USA	HIV Ag ELISA with ICD*[1] (Beckman Coulter)	HIV culture, PCR from PBMC, repeated Ab+ve at >15 months, or seroreversion (detection method not stated)	83	94	23
	Infants 16 days-2 months, plasma, USA			60	100	13
	Infants 2-5 months, plasma, USA			100	100	9
Quinn 1993 [38]	Children <10 yrs, serum, USA	HIV-1 p24 ELISA (Coulter) with ICD	HIV Ab assay (Organon-Teknika) at >15 months of age with symptom assessment	89.5	99.2	404 (158 patients)
Bredberg-Rådén 1995 [39]	Infants 1 week-1.5 yrs, PBMC/plasma, Tanzania	HIV Ag ELISA (Coulter) with ICD	Nested PCR (in-house)	53.0-70.8 (age stratified)	100	69
Bulterys 1995 [40]	Infants 6 weeks and 3 months, plasma, Rwanda	HIV-1 p24 Ag assay with ICD (Abbott)	Western blot at ≥12 months (Bio-Rad) and symptom assessment	15.4	100	220 (36 patients)
Lewis 1995 [41]	Infants <3 months, cord blood or plasma, USA	Ag ELISA (Coulter) with ICD	ELISA and Western blot at 15 months	58	85	46
		NIAID protocol HIV culture		83	100	
		In-house DNA PCR		83	94	

Nielsen 1995 [42]	Infants, <5 yrs, plasma, Brazil	HIV p24 EIA (Abbott) with ICD	ELISA & Western blot > 18 months	With ICD – 71.4 No ICD – 52.4	95	40
Lyamuya 1996 [43]	Infants/adults, plasma/serum, Tanzania	Ag ELISA (DuPont) with ICD and ELAST enhancement	HIVChek (DuPont), Ab HIV-1 ELISA (Wellcozyme, Murex), Western blot (Diagnostic Biotechnology) or PCR (in-house)	97.6	85.8 1st test, 100 overall	507 (453 patients)
Nesheim 1997 [44]	Infants 1 wk-1 yr, PBMC/plasma/serum, USA	HIV p24 ELISA (Coulter) with ICD	Infected: Ab +ve at ≥18 months Uninfected: ≥2x Ab -ve at >6 months PCR on PBMC	35-93.3 (age stratified)	93-100 (age stratified)	345
Panakitsuwan 1997 [45]	Infants 1 day-4 yrs, serum, Thailand	Ag EIA (Coulter) with ICD	Ab by ELISA & gelatin particle agglutination, RT-PCR	With ICD – 85.4/87.8 No ICD – 34.2	100	71
Paul 1997 [46]	Infants <4 months, plasma/serum, USA	HIV-1 p24 ELISA (Coulter) with ICD	HIV culture and symptom assessment	59	100	253 (206 patients)
Rich 1997 [47]	Infants, 0-6 months, plasma/serum, USA	HIV-1 p24 ELISA (Coulter) with ICD	HIV culture, ≥ 2 positive results	27 (<7 days) 77 (1 month) 71-81 (1 to 6 months)	90 (<7 days) 100 (1 month) 98-100 (1 to 6 months)	207 patients
Daar 2001 [48]	Adults, primary HIV, plasma, USA	HIV p24 Ag EIA, (Abbott)	NAAT (Amplicor, Roche or bDNA, Chiron), Western blot (Cambridge Biotech Corp.), or EIA (Abbott)	88.7	100	436
Hecht 2002 [49]		HIV p24 Ag EIA (Abbott)	Genscreen HIV Ultra (Bio-Rad), VIDAS	79	99.5	258

	Adults, primary HIV, plasma, USA	bDNA (Bayer)	Duo Ultra (bioMérieux), VIDAS Duo Quick (bioMérieux), Architect HIV Combo (Abbott), Elecsys HIV Combi (Roche); Genscreen HIV v2 (Bio-Rad), Ortho HIV Capture (Ortho Diagnostic Systems)	100	95.3	
		Amplicor PCR, (Roche)		100	97.3	
		TMA HIV-1 RNA, (GenProbe)		100	98.4	
		Ab-test (Ag Combi EIA, Abbott)		77	96.8	
Weber 2002 [5]	Adults, blood donor panels, plasma/serum/culture supernatants, multi-country.	Cobas HIV combi EIA (Roche)	Various*[2]	100	99.7	>11,000 total across all sites, not all samples tested on all assays
		VIDAS HIV DUO (bioMérieux)		100	100	
		Enzygnost HIV Integral (Dade Behring)		100	99.7	
		Enzymun-Test HIV Combi (Boehringer)		100	100	
		Genscreen HIV1/2, version 2 (Bio-Rad)		98.3	100	
		IMx HIV-1/HIV-2 III Plus (Abbott)		98.7	ND	
		AxSYM HIV-1/2 gO (Abbott)		100	ND	

		Prism Anti-HIV1/2 (Abbott)		ND	99.9	
		Enzygnost Anti-HIV1/2 plus (Dade Behring)		99.8	99.9	
		Cobas Core Anti-HIV 1 2 O EIA (Roche)		98.3	ND	
Sutthent 2003 [50]	Infants 1-6 months, plasma/serum, Thailand	Vironostika HIV Ag/Ab (bioMérieux) with ICD and ELAST	NAAT (Amplicor PCR, Roche)	100	100	471 (391 patients)
Ly 2004 [11]	Worldwide subtype panel, supernatant	6 combined Ag/Ab tests, and 2 Ab-only tests*[3]	HIV-1 Ag MAb assay, HIV-1 p24 Ag quantitation assay (both Abbott) or Cobas core HIV Ag EIA (Roche)	2.5 to >25pg/ml	-	31
	Worldwide samples, plasma/serum			99.8-100	97.2-100	669+ve, 1005 -ve
	Seroconversion panels, plasma			38.1-55.1	-	176 from 25 seroconversion panels
Sherman 2004 [51]	Infants <1yr, plasma, South Africa	HIV-1 p24 ELISA (Perkin Elmer) with ICD and ELAST enhancement	NAAT (Amplicor PCR, Roche)	98.1	98.7	203 (90 patients)
Sickinger 2004 [2]	Adults, Panels,	AxSYM HIV Ag/Ab	Western blot (LAV-blot I & II, Bio-Rad),	100 (Ab)	99.87-99.92	9838

	plasma/serum, multicentre	Combo (Abbott)	HIV Ag Confirmatory (Murex), NAAT (Monitor, Roche)	17.5pg/ml (Ag)		
Respess 2005 [52]	Adult, B-subtype, plasma, USA	HIV-1 p24 ELISA (Coulter) with ELAST enhancement	Versant bDNA assay (Bayer)	46.4 (VL<30,000) 100 (VL >30,000)	100	451 (284 patients)
Nouhin 2006 [53]	Infants 1-24 months, plasma, Cambodia	HIV Ag ELISA (Perkin Elmer) with ICD and ELAST amplification	DNA PCR (in-house) and viral cultures	91.3	100	169 (147 patients)
Patton 2006 [54]	Infants 1 month-12 yrs, DBS, South Africa	HIV p24 Ag ELISA (Perkin Elmer)	NAAT (DNA PCR Amplicor, Roche, or RNA NucliSens, bioMérieux)	98.8	100	141
Yeom 2006 [17]	HIV-positive & seroconversion panels, serum.	LG HIV Ag/Ab Plus ELISA (LG Life Sciences)	Enzygnost HIV Integral (Dade Behring)	100	99.9-100	1282
Fiscus 2007 [55]	Infants <180 days, plasma, USA	HIV p24 Ag ELISA (Perkin Elmer) with ELAST enhancement	NAAT (Roche Amplicor HIV DNA) and/or HIV culture	91.7	98.5	802 (582 patients)
George 2007 [15]	Infants IQR: 0.2 to 3.6 yrs, plasma, Haiti	Vironostika HIV Ag/Ab ELISA (bioMérieux)	NAAT (RNA, NucliSENS EasyQ HIV-1, bioMérieux)	91	97	401 (233 patients)
		Vironostika HIV Ag/Ab		93	99	

		ELISA with ELAST enhancement				
		VIDAS Duo HIV Ag/Ab (bioMérieux)		95	99	
Ly 2007 [56]	See [11]	11 (6) combined Ag/Ab tests; 2 (2) Ab-only tests (from [11]). *3	See [11]	99.4-100	97.2-100	1983
Patton 2008 [57]	Infants 20 days-6 yrs, DBS, South Africa	HIV Ag ELISA (Perkin Elmer) with ELAST enhancement	NAAT (DNA PCR, Amplicor, Roche, or RNA, NucliSENS EasyQ HIV-1, bioMérieux)	88.9-98.3	100	246
Cachafeiro 2009 [58]	Infants, DBS, pan-country	HIV Ag ELISA (Perkin Elmer) with ICD and ELAST enhancement	NAAT (DNA PCR, Amplicor (Roche) or in-house, or RNA NucliSens QT, bioMerieux)	DBS <20 months old: 94.4 (50-100, age stratified)	100	502
				DBS >20 months old: 72.2 (65.6-100, age stratified)		115
Beelaert 2010 [31]	Panels, plasma/serum/whole blood/culture supernatant	Alere Determine Ab/Ag	Various*4	86.6	100	379
		Vironostika HIV Uni-Form II Ag/Ab (bioMérieux)		92.5		

Mwapasa 2010 [59]	Infants 6wks, DBS, Malawi	HIV-1 p24 ELISA (Perkin Elmer) with ICD	HIV-1 DNA PCR (Roche)	84	98	222
Stewart 2010 [60]	Adults, plasma, USA	NAAT (Cavidi ExaVir), HIV-1 p24 ELISA (Perkin Elmer) with ICD and ELAST enhancement +/- modification	NAAT (Amplicor Monitor HIV RNA PCR Roche)	75 with modification, 66 without modification	54 with modification 44 without modification	274 (with modification) or 306 (without modification) (108 patients)
Bhowan 2011 [61]	Adult women, plasma/whole blood, South Africa	Alere Determine Ag/Ab (Inverness)	RT Advance Quality HIV (Intec), Acon HIV-1/2/O Tri-line (Acon)	100 (plasma and whole blood)	99.8 (plasma) 99.3 (whole blood)	1019 (plasma) + 380 (whole blood)
Fox 2011 [34]	Stored serum, p24 Ag+ve, UK	Alere Determine Ab/Ag	EIA for Ag/Ab (VIDAS Duo, bioMérieux) or EIA (Bio-Rad)	50.0	N/A	36
Kivuyo 2011 [62]	Infants, DBS, Tanzania	HIV-1 p24 ELISA (Perkin Elmer)	Amplicor PCR (Roche)	100	95.5	27
Rosenberg 2011 [28]	Adult >18 yrs, whole blood, plasma, Malawi	Determine Ab/Ag (Inverness)	UniGold Recombigen (Trinity Biotech), Bioline HIV 1/2 3.0 (Standard Diagnostics), HIV-1 p24 ELISA (Perkin Elmer) with ELAST enhancement and Monitor HIV RNA PCR (Roche)	0.00 (acute) 99.4 (established)	98.3 (acute) 99.2 (established)	8 acute, 163 established, 838 negative
		HIV-1 p24 ELISA (Perkin Elmer) with ELAST enhancement		71.4 (acute)	100 (acute)	

Spacek 2011 [63]	Adults, non-B, plasma Uganda	HIV p24 ELISA (NEN Life Science) with ICD and ELAST enhancement	NAAT (Amplicor PCR, Roche)		69 overall ($4x10^2$-$5x10^4$c/ml – 45, $5x10^4$-$1x10^5$c/ml – 62, $1x10^5$-$2.5x10^5$c/ml – 68, $2.5x10^5$-$5x10^5$c/ml – 80, >$5x10^5$c/ml – 90)	67	394 (331 patients)
Chetty 2012 [30]	Pregnant women, plasma, South Africa	Determine Ab/Ag (Inverness)	NAAT (PCR, HIV NucliSENSEasyQ v2.0, bioMérieux), Bioline HIV 1/2 3.0 (Standard Diagnostics), SENSA Tri-line HIV-1/2/O (Hitech)		Ag – 3.1, Ab – 59.4	96.9	32
Kilembe 2012 [27]	Adults, acute infection, plasma, Rwanda/Zambia	Determine Ab/Ag (Inverness)	Ag EIA (Beckman Coulter), Vironostika HIV Uni-Form II Ag/Ab, (bioMérieux), Capillus and UniGold (Trinity Biotech)		Ag – 1.9, Ab – 76.7	96.7	82
Patel 2012 [35]	Adults, acute infection, plasma, USA	Architect HIV Ag/Ab Combo (Abbott)	Genetic Systems HIV 1/2 + O (Bio-Rad) or Oraquick (OraSure) or OraQuick Advance (OraSure) or Vironosti	If Ab-ve: NAAT (Aptima HIV-1 RNA Qualitative (Hologic) & Versant HIV-1 RNA 3.0, (Siemens)), retest with HIV 1/2 + O (Genetic	87.8	ND	33
		Determine HIV-1 Ag/Ab Rapid Test (Alere)			75.8		
		Genetic Systems HIV 1/2 + O (Bio-Rad)			57.5		

		Multispot HIV-1/HIV-2 Rapid test (Bio-Rad)	ka HIV-1 MicroELISA (bioMérieux)	Systems) and Multispot HIV-1/HIV-2 (Bio-Rad). If Ab+ve: Western blot (manufacturer not stated)	33.3		
		Clearview Complete HIV 1/2 assay (ChemBio)			29.6		
		Unigold Recombigen HIV (Trinity Biotech)			24.2		
		Clearview HIV 1/2 Stat-pak (ChemBio)			22.6		
		OraQuick Advance Rapid HIV-1/2 Ab (OraSure)			21.9		
Brauer 2013 [33]	Serum, South Africa	Determine Ab/Ag Combo (Alere)	Various*[5]		Ag – 10 Ab - 91	100	79
Faraoni 2013 [29]	Acutely infected adults, serum, Italy	Determine Ab/Ag (Alere)	EIA (Architect HIV-1/2 Ag/Ab, Abbott), NAAT (CAP/CTM, Roche), Western blot (New LAV Blot, Bio-Rad)		Ag – 29.4, Ab – 58.8, Ag or Ab – 88.2	100	17
Tao 2013 [12]	Lysate supernatants (for Ag or p24), seroconversion panels,	Elecsys HIV combi PT (Roche)	Various*[6]		-	99.9	4465
		Elecsys HIV combi (Roche)			-	99.9	675

	NIBSC reference panels, plasma/serum, Korea, China, & Malaysia	Advia Centaur HIV combo (Siemens)		-	99.5	1039
		Architect HIV combo (Abbott)		-	99.8	2751
		Vironostika HIV Uni-Form II Plus O (Abbott)		-	100.0	703
		Zhuhai Livzon Anti-HIV EIA (Zhuhai Livzon Diagnostics)		-	100.0	675
Mühlbacher 2012 [6]	NIBSC Ag standard (90/636)	Elecsys HIV Combi PT	Not applicable	1.05 IU/mL	-	6 dilutions
		Architect HIV Ag/Ab combo (Abbott)		0.94 IU/mL	-	
		Advia Centaur HIV Ag/Ab combo (Siemens)		1.89 IU/mL	-	
		AxSYM HIV Ag/Ab (Abbott)		1.20 IU/mL	-	
	Blood donors	Elecsys HIV Combi PT	Architect HIV Ag/Ab combo (Abbott), Advia Centaur HIV Ag/Ab combo (Siemens), AxSYM HIV Ag/Ab (Abbott), Prism HIV O Plus (Abbott)	-	99.9	7343
	Routine screening samples			-	99.8	4103
	Cross-reactivity samples			-	99.3	296

Salmona 2014 [23]	Adults, serum, France	BioPlex 2200 Ag-Ab (Bio-Rad)	Architect HIV Ag/Ab Combo (Abbott), ImmunoComb II HIV-1 & 2 BiSpot (Orgenics), New Lav Blot I & II (Bio-Rad)	100	99.4	1505
Chang 2015 [9]	Adults, plasma	AxSYM HIV 1/2 gO (Abbott)	New LAV Blot I (Bio-Rad), Cobas Amplicor HIV-1 Monitor v1.5 (Roche)	100	100	152
		HIV (1+2) Ag/Ab (Beijing Wantai Bio-pharm)		98.8	100	
		HIV Combi Ag/Ab EIA (Bio-Rad)		100	98.5	
		HIV Ab/Ag (Dia.Pro)		100	100	
Piwowar-Manning 2015 [21]	>16 years, plasma, whole blood	GS HIV Combo Ag/Ab EIA (Bio-Rad)	Determine HIV-1/2 (Alere), SD Bioline HIV 1/2 v3 (Standard Diagnostics), UniGold HIV (Trinity Biotech), Architect HIV Ag/Ab (Abbott), Aptima HIV-1 RNA (Hologic Gen-Probe)	Established – 100 Acute – 83.3	96.7	612
Urio 2015 [19]	Adults, serum, Tanzania	Enzygnost HIV Integral II Ag/Ab ELISA	Inno-Lia HIV I/II immunoblot (Innogenetics, Belgium)	100	100	600
		Murex HIV Ag/Ab		100	100	
		Vironostika HIV Uniform II Ag/Ab		100	99.5	

Bystryak 2016 [64]	Children, plasma/ serum, USA	ELISA + photochemical signal amplification system	Amplicor HIV-1 Monitor (Roche)	VL <3,000c/mL: 52.6 VL >3,000c/m L: 100	100	182
Kong 2016 [65]	Blood donor panels (early infection), China	xMAP (Luminex, USA)	Western Blot (details not specified), NAAT (RNA, details not specified)	57.8	-	33
Peters 2016 [14]	Adults, acute infection, USA	Architect Ag/Ab (Abbott)	NAAT (Aptima HIV-1 RNA, Gen-Probe, or m2000 RealTime HIV-1, Abbott)	79.8	99.9	168
Stekler 2016 [66]	Adults, acute infection, whole blood/ serum/ plasma/ saliva USA	OraQuick Advance HIV-1/2 (OraSure), saliva	Pooled NAAT (RealTime HIV-1 RNA, Abbott), Architect HIV-1 Ag/Ab (Abbott)	RNA+ve: 75.0 Ag+ve: 85.0	99.9	2180
		OraQuick Advance HIV-1/2 (Orasure), whole blood		RNA+ve: 77.9 Ag+ve: 88.3	100.0	2175
		Uni-Gold (Trinity Biotech)		RNA+ve: 84.9 Ag+ve: 95.7	100.0	1614
		INSTI HIV-1 (bioLytical)		RNA+ve: 73.3 Ag+ve: 84.6	99.8	559
		Determine Ag/Ab Combo (Alere)		RNA+ve: 84.6 Ag+ve: 91.7	99.0	1523
		GenScreen HIV-1/HIV-2+O Ab EIA (Bio-Rad)		RNA+ve: 87.9	99.8	2161

Blaich 2017 [67]	Adults, serum, Switzerland	Elecsys HIV Combi PT (Roche)	VIDAS Duo EIA (bioMérieux), Inno-Lia HIV I/II (Innogenetics), NAAT (Cobas AmpliPrep/Cobas TaqMan HIV-1, Roche)	100	99.7	3997
		Architect HIV Ag/Ab Combo (Abbott)		100	99.8	
Fitzgerald 2017 [68]	Stored and fresh samples, plasma/ serum/ whole blood, UK	HIV-1/2 Ag/Ab Combo (Alere)	Architect HIV Ag/Ab Combo (Abbott), VIDAS Duo EIA (bioMérieux)	p24: 88 Ab: 100	100	120
Ghisetti 2017 [69]	Adults/panels, Italy	Liason XL HIV Ag/Ab (DiaSorin)	Architect HIV Ag/Ab Combo (Abbott), Western blot (New LAV Blot, Bio-Rad), NAAT (Cobas AmpliPrep/Cobas TaqMan HIV-1, Roche)	p24: 9.9pg/mL Ab: 100	99.7	3090
Masciotra 2017 [70]	Adults, stored plasma, USA	Determine HIV-1/2 Ag/Ab Combo	Architect HIV Ag/Ab Combo (Abbott), MultiSpot HIV-1/HIV-2 Rapid (Bio-Rad)	p24: 50.0 Ab: 99.6	100	508
Meggi 2017 [71]	Infants, whole blood, Mozambique	Lynx p24 Ag POC	Cobas AmpliPrep/Cobas TaqMan HIV-1 (Roche), Amplicor HIV-1 Monitor (Roche)	71.9	99.6	879
Stafylis 2017 [72]	Stored serum, USA	Determine HIV-1/2 Combo (Alere)	Advia Centaur HIV-1/O/2 (Siemens), HIV-1 Western blot, Aptima HIV-1 RNA (Hologic), Architect HIV Ag/Ab Combo (Abbott), Cobas AmpliPrep/Cobas TaqMan HIV-1 (Roche)	95	100	133
		SD Bioline HIV Ag/Ab Combo (Standard Diagnostics)		91	100	133

van Tienen 2017 [73]	Adults, stored serum, Netherlands	HIV-1/2 Ag/Ab Combo (Alere)	Architect HIV Ag/Ab Combo (Abbott), Liason XL HIV Ag/Ab (DiaSorin), VIDAS Duo EIA (bioMérieux), Inno-Lia HIV Ab (Inogenetics)	Acute (p24): 65 Recent (p24): 24 Recent (Ab): 100 Chronic (Ab): 100	-	89
Fransen 2017 [74]	Seroconverters (acute), plasma, Belgium	Enzygnost Anti-HIV 1/2 Plus	Inno-Lia Score I/II (Innogenetics), neutralization by Innotest HIV Ag mAb (Fujirebio), NAAT (Cobas AmpliPrep/Cobas TaqMan HIV-1, Roche)	11.4	100	77
		Vironostika HIV Ag/Ab (bioMérieux)		34.3	95	
		Innotest HIV Ag mAb EIA (Fujirebio)		45.7	100	
		Determine HIV-1/2 (Alere)		6.1	-	
		Determine HIV-1/2 Ag/Ab (Alere)		8.6	93.1	
		SD Bioline HIV-1/2 (Standard Diagnostics)		12.1	-	
		SD Bioline HIV Ag/Ab Combo (Standard Diagnostics)		29.4	100	
		Alere HIV Combo		17.1	93.1	
Eshleman 2018 [75]	Adults, acute infection,	Architect HIV Ag/Ab	Oraquick Advance HIV-1/2 Ab (OraSure),	45.8	-	24

	plasma, USA/South Africa	Combo (Abbott)	UniGold Recombigen (Trinity Biotech), Geenius HIV-1/2 Ab (Bio-Rad), NAAT (Aptima HIV-1, Hologic)			
		GS HIV Combo Ag/Ab EIA (Bio-Rad)		50.0		
		BioPlex 2200 HIV Ag-Ab (Bio-Rad)		45.8		
Zhao 2018 [76]	Adults/panels, serum/lysate, China	Lumipulse HIV Ag/Ab (Fujirebio)	Western blot (HIV blot 2.2, MP Diagnostics), NAAT (Cobas AmpliPrep/Cobas TaqMan HIV-1, Roche)	100	99.2	1153
		Elecsys HIV Combi PT		100	100	

Supplementary Table 3. Reported sensitivity and specificity of p24 antigen detection from selected studies. Studies were selected that were designed to comprehensively measure one or more index assay characteristics (e.g. sensitivity, specificity, subtype-breadth) against one or more reference methods.

§ Sensitivity and specificity, where given, are for the Ag detection part of the test unless otherwise stated. Names of companies or assay manufacturers, as far as possible, are given as those at the time of print of the original paper. ELAST enhancement; a tyramide signal amplification system that improves sensitivity for standard horseradish peroxidase-based ELISAs

Abbreviations. Ag: p24 antigen; Ab: antibody; DBS: dried blood spot; ICD: immune-complex disruption; IQR: inter-quartile range; N/A: not applicable; NAAT: nucleic acid amplification test; PBMC: Peripheral blood mononuclear cells; TMA: transcription-mediated amplification.

*1 Two modifications were proposed to the manufacturers guidelines for the assay; the best results of the two modifications are presented.

*2 HIV-1/HIV-2 3rd gen Plus EIA (Genetic Systems), IMx HIV-1/HIV-2 III Plus (Abbott), AxSYM HIV-1/2 gO (Abbott), Prism HIV-O Plus (Abbott), VIDAS HIV DUO (bioMérieux), Genscreen HIV-1/2 (Bio-Rad), Enzygnost Anti-HIV1/2 Plus (Dade Behring), Enzygnost HIV Integral (Dade Behring), Enzygnost Anti- HIV (Dade Behring), Enzymun-Test HIV Combi (Boehringer), and Cobas Core Anti-HIV 1 2 O EIA (Roche Diagnostics).

*3 AxSYM HIV Ag/Ab Combo (Abbott), Enzygnost HIV Integral (Dade Behring), Genscreen Plus HIV Ag/Ab (Bio-Rad), Murex HIV Ag/Ab Combo (Abbott), VIDAS HIV DUO (bioMérieux) and Vironostika HIV Uniform II Ag/Ab (Organon Teknika).

*4 Vironostika HIV Uni-Form (Organon Teknika), HIV1/HIV2 ELISA kit (Cambridge Biotech), Vironostika HIV Uniform II plus O (bioMérieux), the Vironostika HIV Uni-Form II Ag/Ab (bioMérieux) and/or the Enzygnost Anti-HIV 1/2 Plus test (Dade Behring). Reactive samples were further characterized with the Inno-Lia HIV Confirmation assay or the Inno-Lia HIV I/II Score test (Innogenetics)

*5 Cobas HIV Combi kit on Modular E170 (Roche Diagnostics), HIV Ag/Ab Combo kit on AxSYM (Abbott) and HIV Ag/Ab Combo kit on Architect i2000 (Abbott). Two of the HIV-1/2 antibody reactive specimens had confirmatory testing performed on the Determine HIV-1/2 assay (third generation rapid assay) instead of a fourth-generation HIV-

1/2 ELISA. The Cobas HIV Ag kit (Roche) on Modular E170 was used for initial p24 antigen determination on the diagnostic specimens.

*6 Advia Centaur HIV Ag/Ab combo (Siemens), Architect HIV Ag/Ab combo (Abbott), Vironostika HIV Uni-Form II Plus O (bioMérieux), Elecsys HIV combi (Roche), Zhuhai Livzon Anti-HIV EIA (Zhuhai Livzon Diagnostics), Serodia HIV1/2 Particle Agglutination (Fujirebio).

Supplementary Table 4

Reference	Method described	Treatment
Kageyama 1988 [77]	Heat/Acid	200 µl sample mixed with 200 µl glycine-HCl pH 2.0, heated to 70°C for 5/10 min, neutralized with 3 µl 5 M Tris; Tested by Abbott Ag EIA
Mathiesen 1988 [78]	Acid	1 M HCl added to 400 µl serum to give pH 3.0 (usually 30 µl), incubated at RT for 90 min, then 4°C, neutralized to pH 7.4 with 1 M NaOH, immediately used for ELISA
Von Sydow 1988 [79]	Acid	100 µl serum mixed with 25 µl 0.5 M HCl, incubated RT 90 min then neutralized 25 µl 0.5 M NaOH and diluted to 200 µl with PBS. Used in ELISA
Nishanian 1990 [80]		100 µl serum mixed with 50 µl 0.5 N HCl pH 2.5-3.0, incubated 60 min 37°C, neutralized with 50 µl 0.5 N NaOH to pH 7.0. 22 µl Triton X-100 added, then 200µl used for ELISA
Ascher 1992 [81]	Acid	ELISA: 200 µl 1.5 M glycine pH 1.85 added to 200 µl serum, mix, incubate 37°C 60 min, neutralize 1.5 M Tris pH 9.0
Bollinger 1992 [82]	Acid	100 µl specimen mixed 0.5 N HCl, 50 µl incubated 60 min 37°C, neutralized 50 µl 0.5 N NaOH, then ELISA
Papaevangelou 1992 [83]	Acid or Heat	100 µl plasma or serum mixed with 200 µl glycine-HCl pH 2.0, heated to 70°C 10 min. Neutralized to pH 7.4 with 10-20 µl 5 M Tris OR 200 µl plasma or serum titrated with 1 M HCl (usually ~20µl) to pH 3.0, incubated RT for 90 min, then placed on ice and neutralized to pH 7.4 with 1 M NaOH (usually 20 µl)
Chandwani 1993 [37]	2x Acid	100 µl plasma mixed with 190 µl 0.15 M glycine-HCl pH 2.0. Heated 70°C 10 min, neutralized by 10 µl 5M Tris. 200 µl used for ELISA OR 100 µl plasma mixed with 50 µl 0.5N HCl (pH 2-3), incubated 37°C 60min, neutralized 50 µl 0.5 N NaOH on ice
Fenouillet 1993 [84]	Acid	100 µl serum mixed 100 µl glycine pH 2.5 37°C 90min, then neutralized with 100 µl Tris pH 7.5.
Kashala 1993 [85]	PEG or Acid	PEG: 450 µl serum incubated with 350 µl 0.2 M EDTA and 200 µl of 12% PEG for 16-20 h at 4°C. Mix spun at 8,000 xg for 15 min at 4°C. S/n frozen at -70°C; pellet washed 6x in 20x vol of cold PBS, then resuspended in 6 M guanidinium HCl Acid: 100 µl serum incubated with 0.5 N HCl pH 3 1 h 37°C, then neutralized with 0.5 N NaOH. Triton X-100 added, and sample assayed Note: Abs released from disruption also assayed
Lillo 1993 [86]	Acid/Heat	Coulter protocol: 1:1 dilution of serum with 1.5 M glycine-HCl pH 1.8, incubated 90 min 37°C then neutralized with 1.5 M Tris pH 9.0

Miles 1993 [87]	Acid/Heat	Coulter ICD kit: 70 µl sample added to 70 µl 1.5 M glycine-HCl pH 1.8, 37°C 90 min, then 70 µl 1.5 M Tris-HCl pH 7.4, incubate 2 h. 200 µl used for ELISA
Pokriefka 1993 [88]	Heat/Acid	After [80]. 200 µl serum mixed with 100 µl 0.5 N HCl then 37°C 60 min. Adjusted to pH 7 with 0.5 N NaOH, assayed by ELISA
Quinn 1993 [38]	Acid	Not described, but [80] and [82] referenced
Schüpbach 1993 [89]	Heat	Serum or plasma diluted 1:3 with distilled H_2O or 0.5% Triton X-100, 'boiled in dry heat block' (100°C for 2, 3 or 5 min)
Simon 1993 [90]	Acid	Coulter ICD-prep kit. 10 µl lysis reagent, 100 µl sample and 100 µl 1.5 M glycine-HCl pH 1.85 mixed, incubated 37°C for 90 min then 100 µl 1.5 M Tris pH 9 added
Vasudevachari 1993 [91]	Acid or Heat	100 µl serum added to 1.5 M glycine pH 1.8-2.2, incubated 37°C 1 h, then neutralized with 100 µl 1.5 M Tris pH 8.6-9.0 OR 100 µl serum incubated 1 h 50 µl 0.5 N HCl, then neutralized with 50 µl 0.5 N NaOH
Duiculescu 1994 [92]	Acid	100 µl plasma mixed 100 µl 1.5 M glycine-HCl pH 1.85, incubated 37°C for 90 min, then neutralized with 100 µl 1.5 M Tris-HCl; 200 µl used for ELISA
Morand-Joubert 1994 [93]	Acid	100 µl serum added to 190 µl 0.15 M glycine-HCl pH 2, incubated at 70°C for 10 min, neutralized with 10 µl 3.5 M Tris
Schüpbach 1994 [94]	Heat	Samples diluted with 2 volumes of 0.5% Triton X-100, boiled for 5min
Brown 1995 [95]	Acid	ICD-Prep kit, Coulter Immunology
Bulterys 1995 [40]	Acid	100 µl plasma incubated 100 µl glycine and 30 µl lysis buffer, 90 min, 37°C then neutralized with 100 µl Tris. 200 µl then used for ELISA
Gutierrez 1995 [96]	Acid	Coulter ICD; 300 µl serum, 150 µl 1.5 M glycine-HCl, incubated 37°C for 60 min, neutralized with 1.5 M Tris. 200 µl used for ELISA
Kappes 1995 [97]	Acid	Coulter assay, details not given, but see [98]
Lewis 1995 [41]	Acid/Heat	Coulter ICD-prep kit: 100 µl plasma added to 100 µl 0.15 M glycine-HCl with Triton X-100, incubated 90 min 37°C, neutralized with Tris. 200 µl used for ELISA
Nielsen 1995 [42]	Acid/Heat	100 µl plasma added to 190 µl glycine-HCl, then 10 min at 70°C, then 10 µl Tris base. 200 µl used for ELISA
Guay 1996 [99]	Acid	Coulter ICD kit;100 µl sample incubated with lysis buffer and glycine for 90 min, neutralized with Tris, then 200 µl used for ELISA
Lyamuya 1996 [43]	Heat	Samples diluted (1:3, 1:6 or a few 1:12/1:24) with 0.5% Triton X-100, boiled for 5 min at 100°C. 250 µl used for ELISA
Stanojevic 1996 [100]	Acid	100 µl serum incubated with 50 µl 0.5 M HCl for 60 min 37°C, then 50 µl 0.5 M NaOH. Analysed by ELISA
Boni 1997 [101]	Heat	100 µl plasma diluted 500 µl 0.5% Triton X-100, 5 min at 100°C, then ELISA

Nesheim 1997 [44]	Acid	70 µl plasma added to 70 µl 1 M glycine buffer pH 1.85, 21 µl 5% Triton X-100. Incubated 37°C 1 h, then 70 µl 1M Tris pH 9.0. 200 µl used for ELISA (Coulter)
Panakitsuwan 1997 [45]	Acid or Heat	Acid – Coulter ICD-Prep kit Heat – following method of [94]
Paul 1997 [46]	Acid	Samples added to 22.5 µl lysis buffer, then 75 µl glycine, then incubated 37°C 90 min. Then 75 µl Tris, and 200 µl each mixture added to ELISA reaction plate
Rich 1997 [47]	Acid	Coulter ICD_Prep kit, according to manufacturer's instructions
Fackler 1998 [102]	Acid	Not described: Coulter ELISA
Steindl 1998 [103]	Comparison of methods on model samples	Heat - Plasma diluted 1/3 with 7mM SDS/1.5mM DTPA pH 7.2, incubated 95-98°C 4min Heat – Plasma diluted 1/3 with distilled H_2O, incubated 100°C 5min Acid – Plasma diluted 1/3 with 1.5 M glycine-HCl pH 1.85, incubated 37°C 1 h, then neutralized 100 µl 1.5 M Tris-HCl pH 9.0 to 200 µl
Nadal 1999 [104]	Heat	100 µl plasma diluted with 500 µl 0.5% Triton X-100, heated 100°C 5 min, tested by ELISA
Ortigão-de-Sampaio 1999 [105]	Acid	Glycine-HCl (no further details)
Ledergerber 2000 [106]	Heat	100 µl plasma diluted with 500 µl 0.5% Triton X-100, 5 min 100°C.
Read 2000 [107]	Acid	Detail not given, but see [98]
Sutthent 2003 [50]	Heat	Plasma diluted 1:6 with 0.5% Triton X-100, heated 100°C for 5 min
Prado 2004 [108]	Heat	Plasma diluted 1:6 in 0.5% Triton X-100, heated 100°C 5 min. 250 µl used for ELISA
Parpia 2010 [109]	Heat	25 µl plasma mixed with 75 µl (0.67%NP-40 0.2% SDS in PBS), heated 4 min 88°C, then cooled and assayed by dipstick

Supplementary Table 4. Methods explored to disrupt immune complexes of host antibody and p24 in blood, prior to diagnostic analysis. A brief summary of the method, where described, is given.

Supplementary Table 5

Assay	Limit of Detection	Development stage	References
Immune complex transfer enzyme immunoassay	26 fg/mL	No known further development	[110-112]
Immuno-PCR	<1 virion* or 0.2 fg/mL	No known further development	[113,114]
Radioimmunoassay	50 fg/mL	No known further development	[115]
Magnetic immunochromatography	17-33pg/mL	No known further development	[116]
Lateral flow with fluorescence	<1 pg/mL	No known further development	[117]
Lateral flow dipstick	50 pg/mL	Under commercialisation	[109]
Europium nanoparticle–based immunoassay, microchip and biobarcode	0.5 pg/mL 0.1 pg/mL 5 pg/mL	Ongoing, at research stage	[118-121]
Amperometric immunosensor	50 pg/mL 8 pg/mL 0.5pg/mL	No known further development	[122-124]
Capacitive immunosensor.	0.25 fg/mL	Ongoing, at research stage	[125,126]
Amperometric immunosensor	6.4 pg/mL	No known further development	[127]
Immunoliposome PCR	0.24 fg/mL	Ongoing, at research stage. Patent WO2005067583 A2	[128]
Plasmonic ELISA	1 ag/mL	Ongoing, at research stage	[129,130]
Digital immunoassay	4.9 fg/mL 2.5 pg/mL	Commercially available (Quanterix Corp.)	[131-133]
Electrochemiluminescence sandwich immunosensor	1 pg/mL	TBD	[134]
Fluorescent protein array	54 pg/mL	No further development	[135]
Rapid immunofiltration assay	420 pg/mL	Under commercialisation	[136]
Nanoribbon field-effect transistor biosensors	20 fg/mL	TBD	[137]
Thio-NAD cycling amplified ELISA	25ag/mL	TBD	[138]

Photochemical signal amplification system for ELISA	80 fg/mL	Under commercialization, Patent US8916341 B1	[64,139]
Carbon-dot microfluidic immunoassay	20pg/mL	TBD	[140]
Carbon-dot paper ELISA	250pg/mL	TBD	[141]
Zinc nanowire origami biosensor	300fg/mL	TBD	[142]
Colloidal Gold Immunochromatographic Assay	25pg/mL	TBD	[143]
Cytometry bead assay	3.7-30,000pg/mL (subtype dependent)	TBD	[144]
Birefringence ELISA	250pg/mL	TBD. Patent WO2015185504A1	[145]
Magnetic bead ELISA	0.5pg/mL	TBD	[146]
Cantilever with optoplasmonic transduction	0.01-0.5fg/mL	TBD	[147]
Platinum nanocatalyst lateral flow	0.8pg/mL	TBD	[148]
Carbon nanotube/ imprinted polymer-based electrochemical sensor	83fg/mL	TBD	[149]
ELISA with peroxide strip readout	11.6pg/mL	TBD	[150]
Dendrimer nanochain lateral flow	5ng/mL	TBD	[151]
Surface acoustic wave biosensor	48ng/mL	TBD	[152]

Supplementary Table 5: Selected ultrasensitive assays for p24 antigen. fg, femtogram; ag, attogram. Sensitivities or limits of detection given are those calculated by study authors and have not been verified independently. Studies also used a variety of samples including biological buffers or healthy volunteer samples spiked with recombinant p24, or actual patient samples. *Sample volume not reported, TBD, to be determined (for papers from 2014 onwards). Corresponding authors for all papers prior to 2016 were emailed in January 2017 requesting a status update for the studies listed. In the case of invalid email addresses, contact with another author was attempted.

Supplementary Table 6

Biomarker	Observed relationship with p24	References	Summary of findings and study notes
Viral load	Yes	Read 2000 [107]	ICD p24 levels correlated with other biomarker levels (anti-p24 antibody, CD4% and RNA).
		Pascual 2002 [153]	HIV RNA and heat dissociated p24 assay were significantly correlated (R^2=0.6, p<0.0001). The modified p24 assay with heat dissociation step was more sensitive than the unmodified assay.
		Fiebig 2003 [154]	Concurrent increase of HIV-1 RNA and p24 antigen during acute stage of the disease (pre-seroconversion). Slopes (from linear regression of log transformed data) were strongly correlated (R^2 = 0.82).
		Ribas 2003 [155]	Correlation with RNA (Spearman ρ-test, R = 0.751, p<0.0001). p24 assay detected a wider range of subtypes than the viral load assay. p24 outside viral particles has a half-life of 42 days, hence stays temporarily detectable even after viral loads decrease.
		Prado 2004 [108]	Anti-p24 antibody levels have key influence on detection of p24. A significant correlation between HIV RNA and p24 antigen assays was found (p<0.001, β = 0.23; logistic regression), but this was weaker and a more gradual slope for those on a structured treatment interruptions program. p24 assays are not sensitive enough for monitoring in some patients with high CD4 counts.
		Stevens 2005 [156]	Subtype C samples. Moderate correlation between $\log_{10}$ RNA viral load. Major concern is variability and lack of sensitivity.
		Brinkhof 2006 [157]	Evaluated p24 for treatment monitoring in children, and in relation to CD4 levels. Correlation between p24 and RNA (p<0.0001). Statistical support for changes in p24 and RNA with respect to CD4 levels. Study of children.
		Erikstrup 2008 [158]	p24 correlated with RNA level (p<0.0001, R^2 = 0.44).
	No	Coombs 1989 [159]	p24 not as good as plasma viraemia culture counts. No correlation between RNA and p24 or antibody and p24. No immune complex disruption step.

	Unclear	Brown 1995 [160]	Antibody level is important and discordant results occur: (i) p24 can be higher than RNA due to circulating non-virion associated p24 and (ii) antibodies may mask p24.
		Respess 2005 [52]	Correlation with RNA level, though not for viral loads of <5,000c/ml. p24 detection is suitable for paediatric diagnoses, where viral loads are high.
Disease Progression	Yes	Spector 1989 [161]	Used p24 antigen as a virological marker during a trial of zidovudine and ribavirin treatment. Decline in antigenaemia can be used as a biomarker in trials of drugs.
		MacDonnell 1990 [162]	Detectable p24 antigenaemia was strongly associated with more rapid progression to AIDS, regardless of initial CD4 cell count.
		Katzenstein 1992 [163]	Plasma p24 level correlated with 2-tier stage of disease (p<0.001 for disease stage)
		Morand-Joubert 1994 [93]	p24 is an earlier marker of disease progression when used with an immune complex disruption step.
		Bulterys 1995 [40]	Detection of high levels of p24 strongly linked to rapid progression to AIDS and earlier death in babies. Children with lower p24 (<50pg/mL) survived longer (20 months vs 7 months, p-0.02).
		Farzadegan 1996 [164]	Rapid disease progression was more common in people with detectable p24.
		Ledergerber 2000 [106]	Mean p24 increased gradually from early to late stage of the disease. p24 protein level was a significant prognostic factor of survival: RNA (p<0.005) and p24 (p=0.043) both predictors of progression to AIDS.
		Read 2000 [107]	ICD p24 antigen levels were correlated with disease progression better than, or equivalent to, HIV RNA levels or CD4 percent.
		Sterling 2002 [165]	p24 predicted disease progression in early stage HIV especially when combined with information on CD4+ lymphocyte count and HIV viral load. p24 tests (even quantitative tests) were cheaper than viral load or CD4 counts.

		Erikstrup 2008 [158]	p24 was a better predictor of Center for Disease Control category than CD4 count (p<0.001) but a worse predictor of mortality than HIV-RNA and CD4 count.
	Unclear	Baillou 1987 [166]	Variation seen in correlation of p24 antigenaemia with progression to AIDS in different populations. In Europeans, persistent detectable antigen associated with transition to AIDS. In Central Africans, detectable antigen was not found in patients with AIDS.
		Duiculescu 1994 [92]	p24 level decreased during zidovudine treatment. Rapid progression of disease associated with unchanged levels of p24 after treatment. Better information obtained by considering the results from p24 with and without ICD.
		Schüpbach 2005 [167]	Studied p24 dynamics during structured treatment interruptions. p24 responded less rapidly to breaks in HAART than viral load. p24 increases, but not viral load during the first 8 weeks, was inversely correlated to changes in CD4 levels. p24 levels did not always reduce during treatment that successfully reduced viral load.
CD4 Count	Yes	Katzenstein 1992 [163]	Plasma p24 level correlated with CD4 count (p<0.05 for CD4 cell count).
		Duiculescu 1994 [92]	Lower CD4 percent found in those who test positive for p24 antigen levels compared to those who test negative. Study of children.
		Ledergerber 2000 [106]	p24 was found to be a better or equivalent predictor of CD4 depletion than RNA.
		Read 2000 [107]	ICD p24 levels correlated with other biomarker levels (anti-p24 antibody, CD4% and RNA).
		Schüpbach 2005 [167]	Studied p24 dynamics during structured treatment interruptions. p24 responded less rapidly to breaks in HAART than viral load. p24 increases, but not viral load during the first 8 weeks, was inversely correlated to changes in CD4 levels. p24 levels did not always reduce during treatment that successfully reduced viral load.
		Stevens 2005 [156]	Subtype C samples. Highly significant (inverse) correlation between CD4 and p24 level (p<0.0001). Major concern is variability and lack of sensitivity.

		Brinkhof 2006 [157]	Evaluated p24 for treatment monitoring in children, and in relation to CD4 levels. Statistical support for changes in p24 and with respect to CD4 levels. Study of children.
Anti-p24 Antibody	Yes	Duiculescu 1994 [92]	Anti-p24 antibody level was inversely correlated with p24 antigen.
		Read 2000 [107]	ICD p24 levels correlated with other biomarker levels (anti-p24 antibody, CD4% and RNA).

Supplementary Table 6. Summary of studies investigating the relationship between quantitative p24 levels, other biomarkers, and disease progression.

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