Human Immunodeficiency Virus Infections Type 1 Group O: A Review

Infektionen mit HIV-1 Subtyp O: Ein Überblick

G. Hess[1,2]

Abstract: Human immunodeficiency virus (HIV) group O represents a new divergent strain of immunodeficiency viruses of type 1 that have been classified into subtypes A to I and that are summarized under group M. In the past, HIV-1 group O infections have not been recognized reliably by immunoassays, this can now be overcome by the addition of group O specific proteins into the test. The addition of such antigens does not affect the recognition of HIV-1 group M and HIV-2. Modified immunoblots allow also the confirmation of HIV-1 group O infection. The clinical course of HIV-1 group O infection is still uncertain, although cases with full-blown acquired immunodeficiency syndrome (AIDS) have been described. Whether HIV-1 group O infections need a different antiviral therapy than HIV-1 group M infection is open to question. It appears likely that current vaccines to prevent HIV-1 group M need to be modified in the light of HIV-1 group O and its high divergency in the envelope region. Whether additional immunodeficiency viruses or subtypes of current immunodeficiency virus require modification of the current diagnostic, therapeutic and vaccine strategies is so far uncertain.

Keywords: HIV Infections/diagnosis; HIV-1/classification; HIV-1/immunology; AIDS Serodiagnosis; Immunoassay/methods.

Zusammenfassung: Das humane Immundefizienzvirus (HIV) Subtyp O ist ein neuer divergenter Subtyp von HIV-1. In der HIV-1-Gruppe M werden die Subtypen A - I zusammengefaßt. In der Vergangenheit konnten die HIV-1 Gruppe O Infektionen nicht regelmäßig mit Immunoassays nachgewiesen werden. HIV-1 Gruppe O spezifische Antigene, die derzeit in neuen Tests verwendet werden, können die HIV-1 Infektionen nunmehr verläßlich erkennen. Die Verwendung dieser Antigene beeinflußt nicht die Erkennung von HIV-1 Gruppe M und HIV-2 Infektionen. Die HIV-1 Gruppe O Infektion kann durch modifizierte Immunoblots bestätigt werden. Wegen der geringen Zahl der HIV-1 Koinfektionen kann derzeit nicht beurteilt werden, ob der klinische Verlauf von dem von HIV-1 Gruppe M unterschiedlich ist. Gleiches gilt für die antivirale Therapie der HIV-1 Gruppe O Infektion, auch hierüber liegen keine gesicherten Daten im Vergleich zu HIV-1 Gruppe M Infektionen vor. HIV-Vakzine, die derzeit in Entwicklung sind, schützen mit Wahrscheinlichkeit nicht vor einer HIV-1 Gruppe O Infektion, insbesondere wegen der hohen Variabilität und Divergenz in der Envelope-Region. Es ist derzeit offen, ob weitere Subtypen oder weitere HIV-Erreger, die bisher nicht bekannt sind, die derzeitigen diagnostischen, therapeutischen und Impfstrategien in der Zukunft beeinflussen werden.

Schlüsselwörter: HIV Infektionen/Diagnostik; HIV-1/Klassifizierung; HIV-1/Immunologie; AIDS Serodiagnostik; Immunoassay/Methodik.

Very recently highly divergent strains of human immunodeficiency virus type 1 have been identified and have been termed HIV-1 group O [1-5]. Initially, suspicion of human immunodeficiency virus infection was based on clinical symptoms and escaped the recognition of antibody tests to human immunodeficiency viruses type 1 and type 2 [6]. Subsequently, this divergent strain of HIV-1 has been cultured and cloned revealing the basis for the recognition of additional HIV subtypes [5].

This review summarizes the current knowledge on the epidemiology and the recognition of HIV-1 group O infection.

Structure of HIV-1 group O

According to current knowledge, subtypes of HIV-1 termed subtypes A - I have been described with a maximum divergency from the prototype HIV-1 subtype B of 15% [7].

Sequence analysis of the newly recognized group O revealed a sequence divergency from the major HIV-1 subtype A - H of approximately 45% [5]. The sequence divergency between HIV-1 and HIV-2 has been shown to be approximately 60% [8].

Figure 1 shows the phylogenetic tree of the human immunodeficiency virus. As can be seen from this fi-

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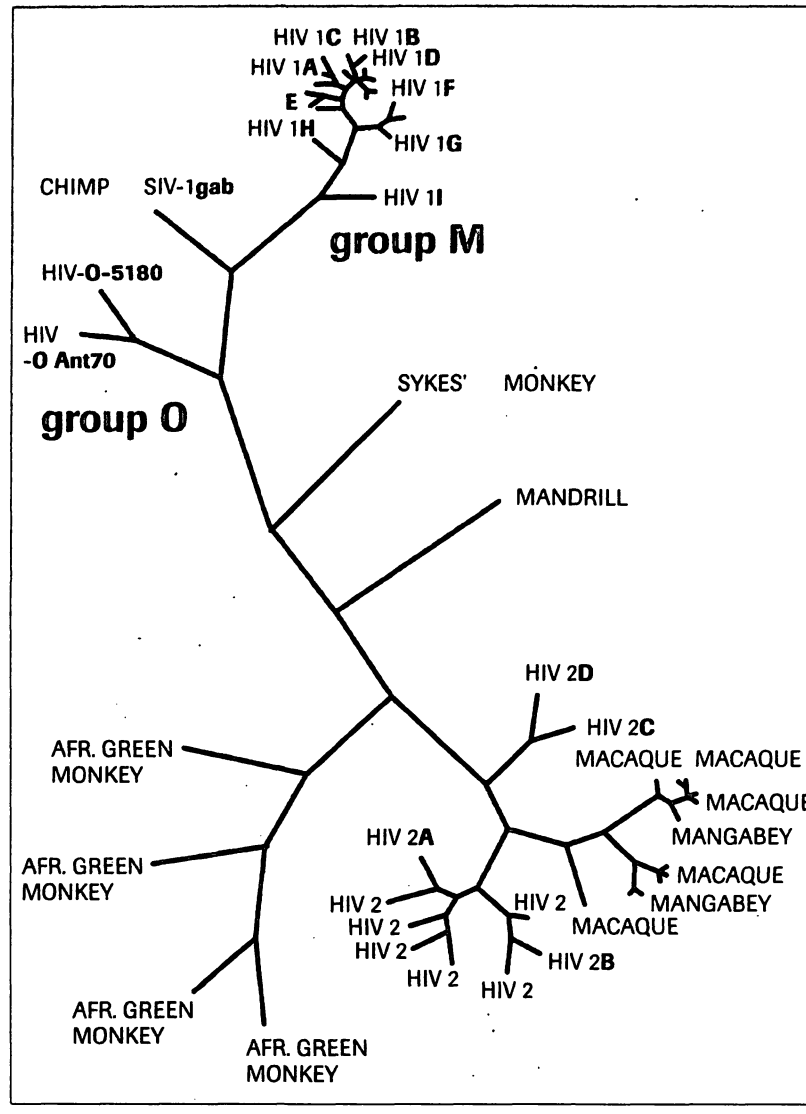

Figure 1 Phylogenetic tree of human immunodeficiency virus type 1

gure, HIV-1 group M and HIV-1 group O isolates can be discriminated. In addition, it has been shown that the gag polyprotein of HIV-1 group M but not of HIV-1 group O binds to cyclophilin and incoporates this cellular peptidyl prolyl-isomerase into virions [9]. In addition, cyclophilin A has been shown to be required for replication of HIV-1 group M but not for HIV-1 group O supporting the phylogenetic diversity of HIV-1 group M and HIV-1 group O [9].

Epidemiology of HIV-1 group O infection

So far, more than 100 HIV-1 group O infections have been identified. This vast majority of HIV-1 group O infections was detected in individuals living in Western Central Africa, coming from countries in Western Central Africa or having had sexual relationships with individuals from Western Central Africa. In this context, HIV-1 group O infections have been described in Belgium and in France [1 - 5] and more recently also in Germany and in the USA. To what extent HIV-1 group O infections have spread to populations without direct or indirect contact to Western Central Africa is so far unknown. Epidemiological studies are in progress to further assess the prevalence

Nonstandard abbreviations: AIDS, acquired immunodeficiency syndrome; CDC, Centers of Disease Control; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus.

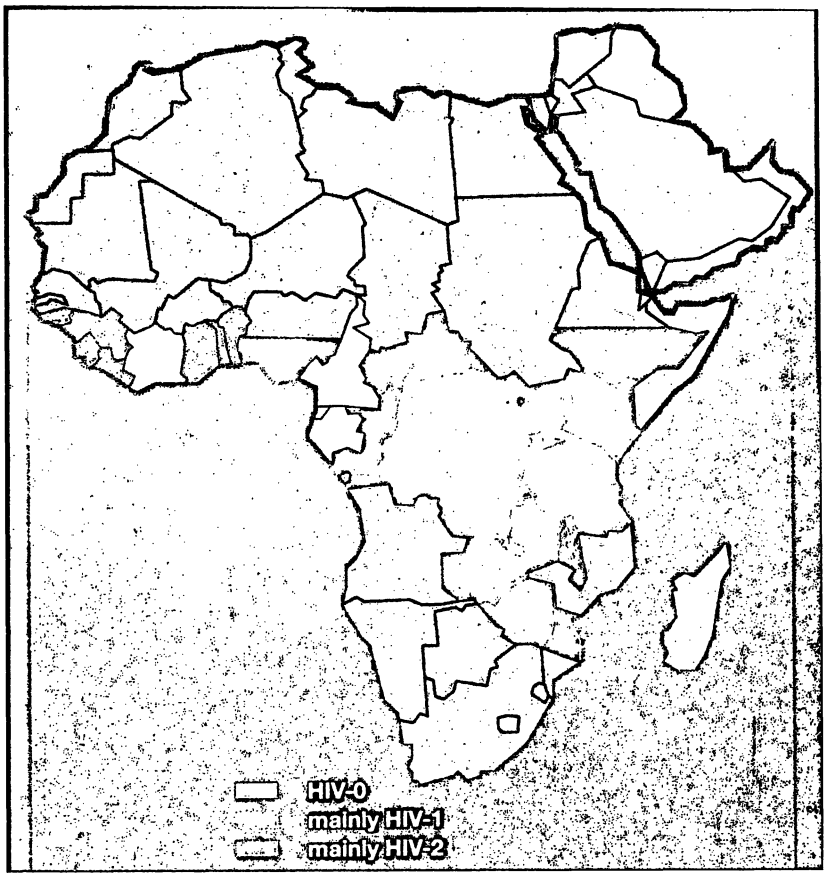

Figure 2 Distribution of immunodeficiency viruses in Africa

of HIV-1 group O infections in Europe and elsewhere.

In Africa, the country with the highest prevalence of HIV-1 group O infection is Cameroon. Approximately 3% of the total population is infected with HIV-1 and roughly 5% of the HIV-1 infected population is believed to be infected with HIV-1 group O [3]. HIV-1 group O is also found in the neighboring countries [1, 4]. Figure 2 shows the distribution of HIV-1, HIV-2 and HIV-1 group O infection in Africa and Figure 3 illustrates the distribution of HIV-1 group M subtypes worldwide.

So far, and this is in contrast to HIV-2 infection, only few co-infections between HIV-1 group M and HIV-1 group O infections have been described (Kaptué, personal communication).

Additional studies are needed to clarify the extent of the distribution of HIV-1 group O in Africa and its distribution to Europe as well as its expansion to other countries around the world.

Clinical features of HIV-1 group O infection

As the number of recognized HIV-1 group O infections is still limited, a clear picture as to the disease association cannot be made presently. The majority of cases that have been identified as HIV-1 group O infection, has presented with symptoms or infections that can be characterized as full-blown AIDS (CDC stage IV). Also asymptomatic individuals have been found [1 - 6]. So far, it remains uncertain whether the clinical course of HIV-1 group O infection is similar to HIV-1 group M infection. Longterm follow-up studies are needed to identify more closely the natural course of HIV-1 group O infection in comparison to HIV-1 group M infection.

There is insufficient data as to the response of HIV-1 group O infection to antiviral therapy. The divergency of the HIV-1 group O envelope protein in comparison to the HIV-1 group M protein renders it

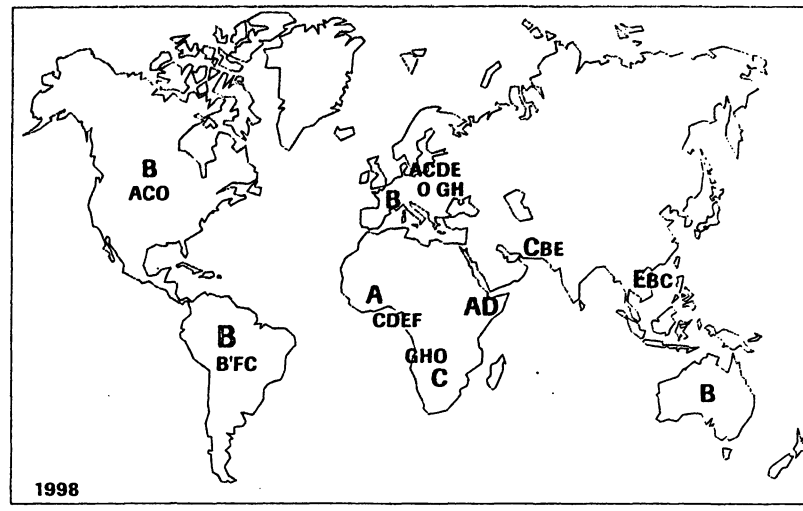

Figure 3 Detection of HIV-1 group M subtype worldwide

difficult to utilize vaccines that are currently under development.

Diagnosis of HIV-1 group O infection

a) Screening

Due to the high divergency of HIV-1 group O to HIV-1 group M conventional anti-HIV 1/2 tests did not always recognize HIV-1 group O infections when the anti-HIV test was used as a screening test [10, 11].

Recently, the addition of a HIV-1 group O specific envelope protein to the anti-HIV-1 and 2 test revealed a significant improvement in the recognition of HIV-1 group O infections. As shown in Figure 4 a recently developed anti-HIV 1/2 and O test by Boehringer Mannheim featured, in addition to an antigen specific for HIV-1 group O, a modified test principle to recognize immunoglobulin M antibodies and HIV-antigen (p24). Using this newly developed screening test for immunodeficiency viruses, all 27 HIV-1 group O infections were recognized, and in addition, HIV-1 and HIV-2 infections were detected in all the positive samples. Moreover, seroconversion samples were recognized approximately 1.5 days earlier than with a HIV-1 and 2 test previously distributed by the same manufacturer [12, 13]. This indicates that an anti-HIV test can be adapted to new scientific knowledge and can improve the recognition of new immunodeficiency viruses.

b) Confirmation of a positive ELISA test result

A positive anti-HIV 1/2/O test is confirmed by Western blot. In the past, the majority of HIV-1 group O infections has shown positive bands on Western blot due to the cross reactivity on the gp 160, gp 41, the reverse transcriptase and the integrase. Also a cross reactivity on the core protein was recognized [14]. A minority of HIV-1 group O infections, however, revealed a negative Western blot suggesting a false positive result of the initial screening test, although the patient was HIV-1 group O infected [1].

The detection of HIV-1 group O infections therefore requires novel immunoblots in order to allow a reliable confirmation of a positive screening result due to HIV-1 group O infection. Such a Western blot is shown in Figure 5. Recombinant ANT70 or V3 loop antigens can be added to the conventional Western blot and thereby give reliable information as to the presence of HIV-1 group O infection, and even allow further characterization of such sera.

c) Detection of nucleic acid in patient sera

Polymerase chain reaction is a widely used amplification procedure in order to amplify human immunodeficiency virus nucleic acid in sera or cells. The PCR technology, however, amplifies reliably only HIV-1 group M. Therefore, in order to recognize HIV-1 group O, polymerase chain reaction specific primers are needed for amplification of HIV-1 group O. Table 1 shows primer sequences of different regions of HIV that allow selective amplification of HIV-1 group M and HIV-1 group O infections.

Test algorithm to identify HIV 1 and HIV 2 infection

According to the present knowledge, sera are screened by a ELISA recognizing HIV-1, HIV-2 and HIV-1 group O infections using proteins of all distinct viruses. Sera that read positive in the initial screening assay can then be further discriminated in differential ELISA tests specific for HIV-1, HIV-2 or HIV-1 group O. Such specific ELISAs will give also evidence of double infections, for example of HIV-1 group O with HIV-1 group M immunodeficiency viruses as has been shown in the past with HIV-1 and HIV-2 [15].

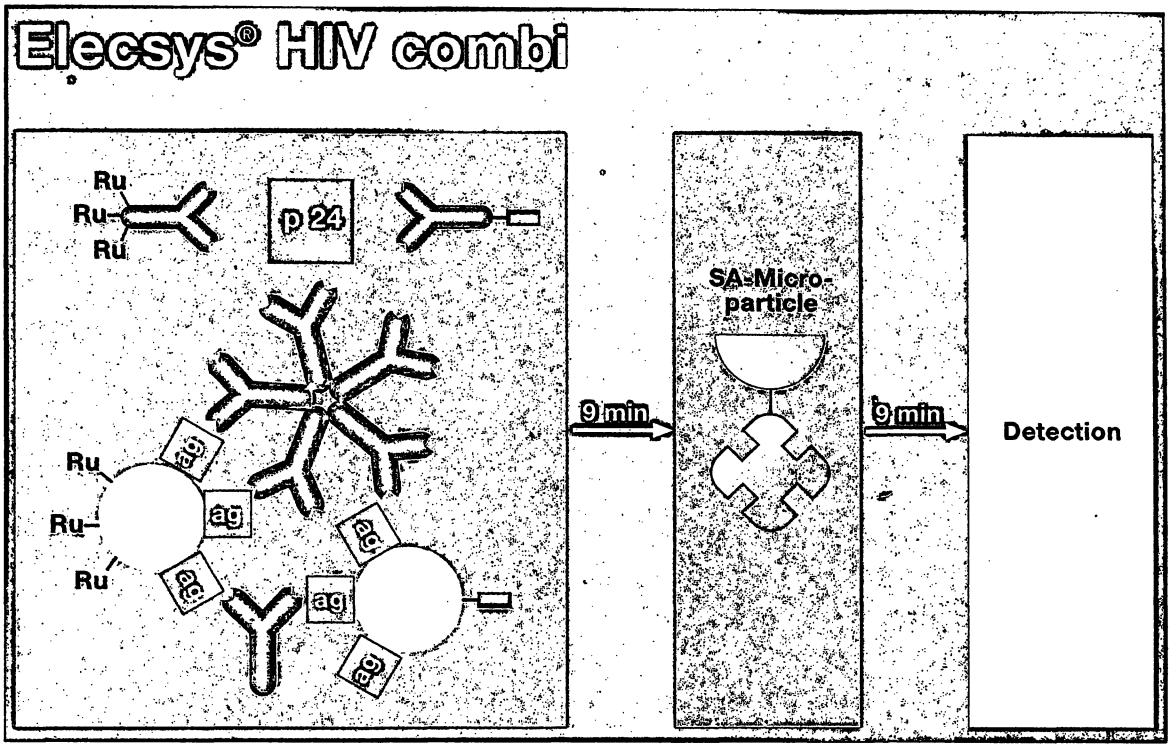

Figure 4 By testing for the p24 antigen and HIV antibodies (IgG or IgM) simultaneously, biotinylated and Ruthenium-labeled monoclonal antibodies to p24 and HIV antigens are incubated with human serum. After a 9 minute incubation period streptavidin-coated micro-particles are added. Immune complexes are detected by electrochemiluminescence reaction.

- Ruthenium-labeled HIV-specific antigens bound to a carrier
- Biotinylated HIV-specific antigens bound to a carrier
- IgG-HIV antibody
- IgM-HIV antibody
- Ruthenium labeled monoclonal p24-antibody
- Biotinylated monoclonal p24-antibody
- p24-antigen

Table 1 Primers used for the differentiation of HIV-1 and HIV-1 group O

HIV-1		
GAG	gaga:	CTACTAGTAC CCTTC AGG
	gagb:	CGGTC TACATAGTCT CTAAAG
nested	sk38:	CCACC TATCC CAGTA GGAGA A
	sk39:	CCTTT GGTCC TTGTC TTATG TCCAG AATG
POL	pol3:	TGGGA AGTTC AATTA GGAAT ACCAC
	pol4:	CCTAC ATACA AATCA TCCAT GTATT G
nested	pol3n:	TGGAT GTGGG TGATG CATA
	pol4n:	AGCAC ATTGT ACTGA TATCT A
ENV	enva:	TGTTC CTTGG CTTCT TG
	envb:	GAGTT TTCCA GAGCA ACCCC
nested	sk68:	AGCAG CAGGA AGCAC TATGG
	sk69:	GCCCC AGACT GTGAG TTGCA ACAG

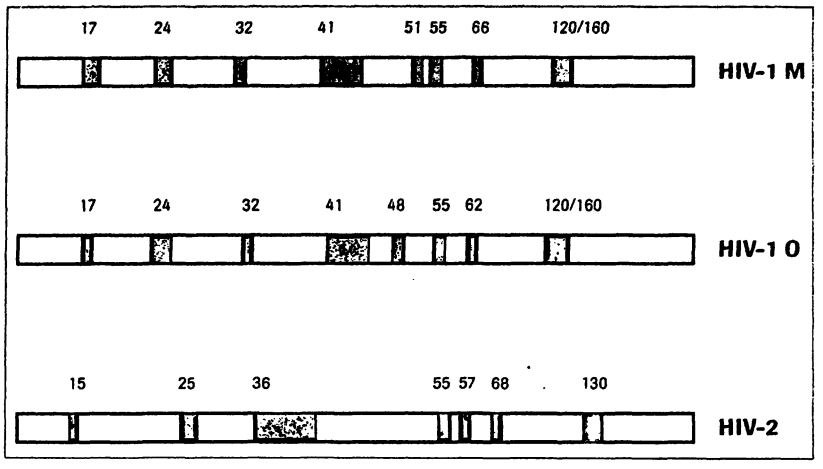

Figure 5 Western blot test for the confirmation of HIV-1 group O infections

Specific immunoblots to confirm HIV-1 and HIV-2 infection have been developed in the past. The addition of an HIV-1 group O specific band using the ANT70 protein is needed for identification of an HIV-1 group O infection.

Detection of nucleic acid by polymerase chain reaction or other amplification techniques, virus isolation by conventional methods and sequencing of parts of the amplified product, mainly the V3 loop can be used to obtain additional information on the immunodeficiency virus detected.

Discussion

According to our present knowledge, Africa appears to be the homeland of immunodeficiency viruses. All major strains of HIV-1 group M were found in Africa, in addition, in parts of Africa HIV-2 and HIV-1 group O are present [1 - 11]. While HIV-1 group M has spread to Europe and other parts of the world, the spread of HIV-2 and HIV-1 group O is still restricted mainly to European countries with a close link to Africa [1 - 11]. At present, it is unclear whether HIV-1 group O remains the last newly recognized human immunodeficiency virus from the African continent. Because of the poor infrastructure, it is difficult to identify candidates that might suffer from full-blown AIDS which could be an indicator of an immunodeficiency virus infection so far not recognized.

Due to its close links to Africa, there is strong concern mainly in Europe that immunodeficiency virus infections could spread from Africa to Europe and then be transmitted by blood or blood products, by drug abuse or sexual contacts. Therefore, manufacturers of HIV-1 and 2 tests have recently adapted their screening tests also to the recognition of the HIV-1 group O [14, 15].

So far, the number of recognized HIV-1 group O infections that have been recognized is still limited. They might not give a sufficient data base for optimization of current anti-HIV 1/2/0 tests for the detection of HIV-1 group O. In addition, tools are now available to identify individuals who might have double infections with HIV-1 group M and HIV-1 group O or even HIV-2 and HIV-O. It needs to be seen whether the clinical course of HIV-1 group O infection differs significantly from HIV-1 group M infection.

Depending on the extent of the spread of HIV-1 group O infection, and on pilot studies with antiviral therapies, it needs to be decided whether drug regimens need to be developed. A major concern comes presently from the assumption that HIV-1 group O might escape current vaccines against HIV that are under development. Such vaccines consist of envelope proteins normally of the predominant HIV-1 subtype in Europe and the US which is HIV-1 subtype B. It needs to be decided whether the vaccine requires modification or alternatively, which appears however to be not desirable, a new vaccine needs to be developed to protect from HIV-1 group O infection, too.

In summary, the recent recognition of HIV-1 group O infection in Africa and parts of Europe has strengthened the diagnostic efforts to recognize all immunodeficiency virus infections. According to our present knowledge, this goal has been met; it is open to question at the present time, whether additional human immunodeficiency virus infections will be found that require further modifications of current diagnostic approaches.

References

1. Gürtler LG, Zekeng L, Simon F, Eberle J, Tsague JM, Kaptue L, Brust S, Knapp S.Reactivity of five anti-HIV-1 subtype O specimens with six different anti-HIV screening ELISAs and their immunoblots. J Virol Methods 1995;51:177-84.
2. Clavel F, Guétard D, Brun-Vézinet F, Chamaret S, Rey AM, Santos-Ferreirao, Laurent AG, Dauguet C, Katlama C, Rouzioux C. Klatzmann D, Champalimaud JL, Montagnier L. Isolation of a new

human retrovirus from West African patients with AIDS. Science 1986;233:343-6.
3. Gürtler LG, Hauser PH, Eberle J, von Brunn A, Knapp S, Zekeng L, Tsague JM, Kaptue L. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. J Virol 1994;68:1581-5.
4. Nkengasong JN, Peeters M, vanden Haesevelde M, Musi SS, Willems B, Ndumbe PM, elaporte E, Perre JL, Piot P, van der Groen G. Antigenic evidence of the presence of the aberrant HIV-1 ANT70 virus in Cameroon and Gabon. AIDS 1993;7:1536-7.
5. Vanden Haesevelde M, Decourt JL, De Leys RJ, Vanderborght B, van der Groen G, van Heuverswijn, Saman E. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency isolate. J Virol 1994;68:1586-96.
6. Loussert-Ajaka I, Ly TD, Chaix ML, Ingrand D, Saragosti S, Couroucé AM, Brun-Vézinet F, Simon F. HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients. Lancet 1994;343:1393-4.
7. Louwagie J, McCutchan FE, Peeters M, Brennan TP, Sanders-Buell E, Eddy GA, Van der Groen G, Fransen K, Gershy-Damet GM, Deleys R, Burke DS. Phylogenetic analysis of gag genes from 70 international HIV-1 isolates provides evidence for multiple genotypes. AIDS 1993;7:769-80.
8. Eberle J, Loussert-Ajaka I, Brust S, Zekeng L, Hauser PH, Kaptue L, Knapp S, Damond F, Sragosti S, Simon F, Guertler LG. Diversity of the immunodominant epitope of gp41 of HIV-1 subtype O and its validity for antibody detection. J Virol Methods 1997;67:85-91.
9. Braaten D, Franke EK, Luban J. Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIV (CPZ) GAB but not group O HIV-1 or other primate immunodeficiency viruses. J Virol 1996;70:4220-7.
10. Gürtler LG, Eberle J, Lorbeer B, Deinhardt F. Sensitivity and specificity of commercial ELISA kits for screening anti-LAV/HTLV-III. J Virol Methods 1987;15:11-23.
11. Artenstein AW, Coppola J, Brown AE, Caqrr JK, Sanders-Buell E, Galbarini E, Mascola JR, VanCott T, Schonbrood P, McCutchan FE, Burke DS. Multiple introductions of HIV-1 subtype E into the western hemisphere. Lancet 1995;346:1197-8.
12. Gürtler L, Michl U, Mühlbacher A, Hofmann H, Heinz F, Paggi G, Bossi V, Thorstensson R, Villaescusa R, Eiras A, Hernandez JH, Weber B. Reduction of the diagnostic window period with a new combined p24 antigen and human immunodeficiency virus antibody screening assay. J Virol meth 1998 (in press).
13. Weber B, Bargane Fall EHM, Berger A, Doerr HW. Reduction of the diagnostic window period with new fourth generation human immunodeficiency virus screening assays. J Clin Microbiol 1998 (in press).
14. Weber B. Multicenter Evaluation of the new automated enzymun-test Anti-HIV 1 + 2 + subtype O. J Clin Microbiol 1998;36:580-4.
15. Hess G, Babiel R, Thongcharoen P, Greenspan J, Michl U, Melchior W, Faatz E. Evaluation of a novel enzymune assay for the detection of antibodies to human immunodeficiency virus types 1 and 2 including subtype O of HIV-1. Clin Lab 1998;44:427-33.

ECLM SYMPOSIUM

November 19, 1998

POCT

Perspectives of point-of-care testing

Program

Perspectives of point-of-care testing
Point-of-care testing (POCT) threatens central laboratories. Therefore it appears essential that all laboratories are aware of the present developments. A prognosis has been published that in a few years about 80% of laboratory testing will be performed near the patient:

- Does POCT reverse centralization of laboratory services?
- Can rapid response testing prevent the POCT trend?
- How should central laboratories react to this trend?
- What are the plans of industry in the face of a new promising market?
- How will quality assessment and accreditation of POCT be performed?

1. Survey of present POCT activities	*H. Schlebusch*	14.30 -15.00
2. Automation concepts of POCT	*R. Felder*	15.00 -15.30
Commercial examples		15.30 -16.30
3.1 Opti Critical Care Analyzer		*H. Merkle,* AVL
3.2 Decentral testing systems: a contribution to improve the economics of critical care		*B. Herpichböhm,* Roche Diagnostics
3.3 The importance of information management in a quality POCT testing program		*J. Tordella,* Chiron
3.4 Performance of a new diagnostic future with I-STAT		*J. Hoelper,* Hewlett Packard
Break		16.30 -16.50
4. Future capabilities of POCT	*W. Wahlefeld*	16.50 -17.10
5. National recommendations for POCT	*O. Müller-Plathe*	17.10 -17.30
6. Round table discussion:		17.30 -18.00
POCT versus rapid response testing by total laboratory automation		*R. Haeckel, 0. Müller-Plathe*

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