Screening of HIV-1 Infection: Epidemiological Data from NARCSG for Greece, 2022

Maria Androutsopoulou†
Graduate Student
Department of Biomedical Sciences
National AIDS Reference Center of Southern Greece,
University of West Attica
ma.androutsopoulou@gmail.com

Klaudia Milaj†
Graduate Student
Department of Biomedical Sciences
National AIDS Reference Center of Southern Greece,
University of West Attica
klaudiamilaj@gmail.com

†These authors contributed equally to this work.

Approved in July 2024

Περίληψη **– Η λοίμωξη από τον ιό HIV παραμένει μέχρι και σήμερα σημαντικό πρόβλημα δημόσιας υγείας με 4.000 άτομα να μολύνονται καθημερινά σε παγκόσμιο επίπεδο. Σύμφωνα με τον Παγκόσμιο Οργανισμό Υγείας, το 2022 υπήρξαν 1,3 εκατομμύρια νέες HIV λοιμώξεις, συνολικά 39 εκατομμύρια άτομα ζούσαν με τον ιό, ενώ 630.000 άνθρωποι απεβίωσαν από ασθένειες που σχετίζονται με τον ιό HIV και τις επιπλοκές του. Με βάση τα δεδομένα του Εθνικού Οργανισμού Δημόσιας Υγείας (ΕΟΔΥ), στην Ελλάδα τα έτη 2021 και 2022 διαπιστώθηκαν 526 και 565 νέες λοιμώξεις αντίστοιχα. Έχει αποδειχθεί πως η όσο το δυνατόν πιο έγκαιρη διάγνωση, καθώς και η χορήγηση και λήψη κατάλληλης αντιρετροϊκής θεραπείας, παίζει καθοριστικό ρόλο στην εξέλιξη της λοίμωξης και, κατά συνέπεια, στη διατήρηση της ποιότητας ζωής των ατόμων που ζουν με τον ιό. Σήμερα, ο αρχικός εργαστηριακός έλεγχος γίνεται με μεθόδους ΕΙΑ 4ης γενιάς υψηλής ευαισθησίας, ενώ ο επιβεβαιωτικός έλεγχος περιλαμβάνει επιπλέον ανοσολογικές εξετάσεις υψηλής ειδικότητας, με συνηθέστερες την Western blot και το Geenius. Στο παρόν άρθρο παρουσιάζονται δεδομένα που αφορούν όλες τις νέες διαγνώσεις HIV που πραγματοποιήθηκαν στο Εθνικό Κέντρο Αναφοράς AIDS Νοτίου Ελλάδος (ΕΚΑΑΝΕ) κατά την διάρκεια του 2022. Συγκεκριμένα το έτος 2022 στο ΕΚΑΑΝΕ επιβεβαιώθηκαν 288 νέες λοιμώξεις. Η πλειοψηφία των επιδημιολογικών δεδομένων που προέκυψαν συμφωνεί με τα, μέχρι τώρα δημοσιευμένα, νεότερα ευρωπαϊκά και ελληνικά επιδημιολογικά δεδομένα που αφορούν την HIV-λοίμωξη.**

Λέξεις κλειδιά: Αρχικός έλεγχος, Επιβεβαιωτικός έλεγχος, Νέες διαγνώσεις, ιός ανθρώπινης ανοσοανεπάρκειας

Apostolos Beloukas
Associate Professor
Department of Biomedical Sciences
National AIDS Reference Center of Southern Greece,
University of West Attica
abeloukas@uniwa.gr

Panagiota Resta
Molecular Biologist and Geneticist
National AIDS Reference Center of Southern Greece
University of West Attica
presta@uniwa.gr

Kassandra Procter
PhD candidate
Department of Biomedical Sciences
University of West Attica
kprocter@unwa.gr

Marika Kotsianopoulou
Assistant Professor
Department of Public Health Policy
National AIDS Reference Center of Southern Greece University of West Attica
mkotsianopoulou@uniwa.gr

Vasileios Papavasilopoulos
Professor
Department of Public Health Policy
National AIDS Reference Center of Southern Greece
vpapavasilopoulos@uniwa.gr

Georgina Tzanakaki
Professor
Department of Public Health Policy
University of West Attica
National AIDS Reference Center of Southern Greece
gtzanakaki@uniwa.gr

Summary **– HIV infections remain a major public health problem with 4,000 people being infected every day worldwide. According to the World Health Organization in 2022, 1.3 million new HIV infections were recorded globally, 39 million people were living with HIV, and 630,000 people died from HIV-related diseases. In Greece, the local CDC reported 526 and 565 new infections for the years 2021 and 2022, respectively. Early diagnosis and proper antiretroviral treatment have been proven to play a decisive role in the progression of the infection and, consequently, in maintaining the quality of life of people living with HIV. Nowadays, initial screening is conducted using highly sensitive 4th generation EIA methods, and confirmatory testing involves highly specific immunological assays, with Western blot and Geenius being the most commonly used. This article presents data concerning all the new diagnoses of HIV infection carried out at the National**

AIDS Reference Center of Southern Greece (NARRCG) during 2022. Specifically, 288 new cases were confirmed at NARCSG. The majority of the epidemiological data obtained agree with the most recently published European and Greek epidemiological data on HIV.

Keywords: HIV, AIDS, Screening test, Confirmatory test, New diagnoses

I. INTRODUCTION

Human immunodeficiency virus (HIV) belongs to the family Retroviridae within the genus Lentivirus. HIV is the causative agent of Acquired Immunodeficiency Syndrome (AIDS), which represents the final stage of HIV infection.

The virus targets CD4+ T-lymphocytes, play a crucial role in the immune system and, consequently, in the immune response. There are two main types of the virus: HIV-1, responsible for the majority of epidemics worldwide, and HIV-2, primarily found in West Africa (1, 2). HIV remains a significant public health problem, with a total of 85.6 million infections and 40.4 million deaths worldwide by the end of 2022. During this period, 39 million people were living with HIV, 1.3 million people were newly infected (46% of whom were women), and 630,000 deaths were caused by HIV-related illnesses (3, 4).

HIV is transmitted through bodily fluids such as blood, vaginal, seminal, and pre-seminal fluids, and breast milk (5). The most common mode of transmission is through unprotected sexual contact. Sharing needles and syringes among people who inject drugs is also a significant mode of transmission. Additionally, mother-to-child transmission can occur during pregnancy, delivery, or breastfeeding (6, 7). High-risk groups identified by UNAIDS include people who inject drugs (PWID), sex workers, men who have sex with men (MSM), transgender people and prisoners (8). However, it is worth mentioning that when people living with HIV achieve an undetectable viral load through antiretroviral therapy (ART), they cannot transmit the virus to others through sexual contact (Undetectable = Untransmittable) (9, 10).

The epidemiological data presented here emphasize the significance of screening and the early detection of HIV infection. To achieve this goal, the National AIDS Reference Center of Southern Greece (NARCSG) follows a specific algorithm based on national and global guidelines for screening and confirmation of HIV test results. Specifically, the Greek CDC recommends the usage of fourth-generation immunoassay as the initial screening test, based on international and European guidelines for simultaneous detection of HIV-1/2 IgM and IgG antibodies and HIV-1 p24 antigen. If the initial screening test is positive, a confirmatory test is also performed. In cases where the result is negative or indeterminate, there remains a possibility of acute infection or a false-positive screening test result. Therefore, re-examination is recommended after 14 days. If the initial screening test result is negative, a repetition of testing is not necessary (11).

II. MATERIAL – METHODOLOGY

The purpose of this study was to detect and conduct epidemiological surveillance of HIV-1 infection in Greece for the year 2022, utilizing data concerning new diagnoses made at NARCSG.

Testing for HIV-1 infection was performed on samples sent to NARCSG from various health facilities (hospitals, private diagnostic laboratories, etc.) throughout 2022. While some rare cases involved samples sent to NARCSG for initial testing (without prior screening), most samples were sent for confirmation of HIV infection after an initial positive screening test. Acceptable samples for diagnostic testing were serum and plasma.

A. ELISA - screening test

The fourth-generation ELISA, Genscreen® HIV-1/2 Ag/Ab (Bio-Rad), used at NARCSG, is widely accepted as a gold standard method for the initial screening of HIV infection due to its very high sensitivity. Both recombinant antigens and monoclonal antibodies are immobilized on the solid phase of fourth-generation ELISAs to simultaneously detect IgG and IgM antibodies and the p24 antigen (13).

B. WESTERN BLOT - confirmatory test

Western blot is used as the confirmatory method for ELISA-positive samples. At NARCSG, both the New Lav Blot I (Bio-Rad) and RecomLine HIV-1/2 IgG (Mikrogen) are employed to confirm HIV-1 infection. These kits contain ready-to-use nitrocellulose strips where recombinant specific viral antigens of both HIV-1 and HIV-2 have been electrophoretically transferred. In the New Lav Blot I Assay, enzyme-conjugated anti-human antibodies are labeled with alkaline phosphatase, whereas in the RecomLine HIV-1/2 IgG Assay, the antibodies are labeled with peroxidase.

C. GEENIUS - confirmatory test

The HIV-1/2 Geenius Differential Test is a rapid test approved by the Food and Drug Administration (FDA) for confirming positive results from fourth-generation ELISA screening tests (12, 14, 15). This assay uses two-level lateral flow immunochromatography cassettes for detecting antibodies against synthetic or recombinant antigens of both HIV-1 and HIV-2 (16). This particular method is superior to other HIV confirmatory tests because it is less complex (three-step process), requires less analysis time (totally 30 minutes), it has the ability to differentiate between HIV-1 and HIV-2, and also is able to interpret the result with the help of an automatic reader (Geenius Reader) minimizing the errors due to subjectivity in the evaluation of the results (12, 15, 16, 17).

III. RESULTS

In 2022, a total of 711 HIV-1 detection tests were performed at the NARCSG. Of these, 54.3% were negative for HIV-1, 4.7% were indeterminate, and 41% were found positive. Based on these positive results, 288 new diagnoses were made. The highest number of tests per month was

conducted in January 2022, while the fewest were performed in December 2022.

The average number of new diagnoses per month was 24, with the month November having the highest number at 29 new cases. This was followed by March and July with 28 new diagnoses each, February and June with 27 each, April with 26, October with 24, and May with 20. The fewest monthly diagnoses were observed in August and December, with 17 and 15 positive results, respectively (Figure 1).

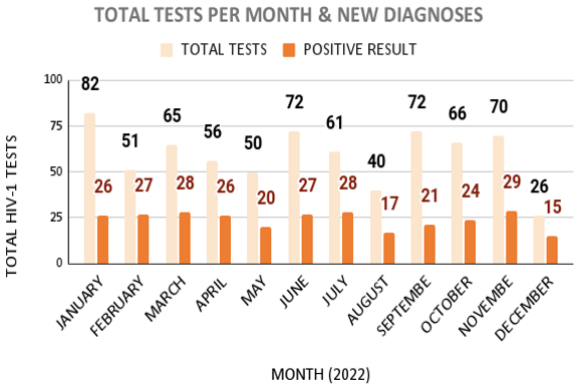

Figure 1. *In the first diagram (up), the total HIV tests and the total tests with positive result are presented, while in the second diagram the same data are categorized per month. (Data from NRCASG, 2022).*

Geenius was the most frequently used confirmatory method at NARCSG in 2022, confirming 57% of new diagnoses (164 samples). Western blot was used to confirm 119 samples. Additionally, 5 individuals were confirmed positive using molecular testing (Real-time RT-PCR). There were 31 cases with indeterminate results, with 15 related to Western blot and 16 to Geenius.

The majority of positive results were from the Attica region. Additionally, 25 cases were reported from the islands, and 6 from the rest of Southern Greece. Among the 25 newly diagnosed cases on the islands, 15 were individuals residing in migrant accommodation centers in Mytilene and Samos (Figure 2).

In terms of their origin, 223 (77%) out of the 288 individuals diagnosed at NARCSG were Europeans, 46 (16%) were Africans, and 20 (7%) were Asians or Americans. The majority of diagnosed individuals were men between the ages of 30 and 40. The most common age group was 31-40 years, comprising 85 (30%) of all cases (Figure 3).

It is important to note that the information presented was obtained through an analysis of the accompanying sample forms and may not necessarily represent a complete or entirely accurate account of all factors contributing to HIV transmission. Nonetheless, these data provide valuable insights into the epidemiology and risk factors associated with HIV transmission in this population.

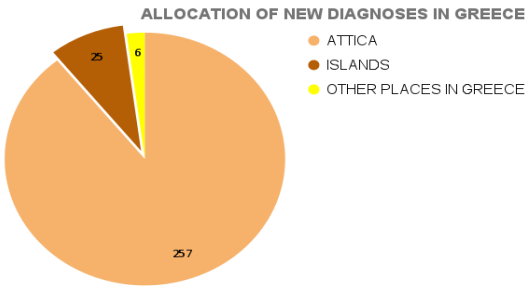

Figure 2. *Allocation of total new diagnoses in Greece (Data from NRCASG, 2022).*

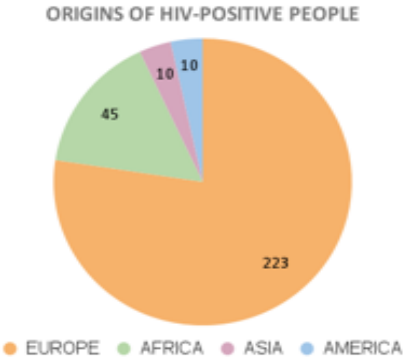

Figure 3. *The pie graph represents the origins of HIV-positive people (up), while the other two-line graphs represent the gender and the age of them (Data from NRCASG, 2022).*

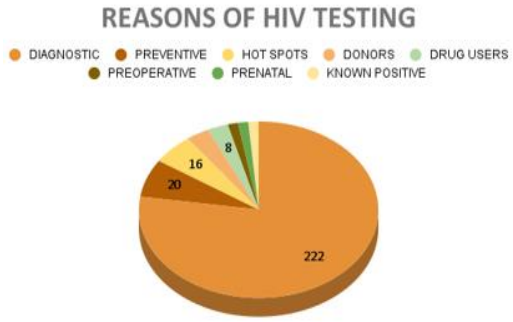

Figure 4. The different reasons of HIV testing (Data from NRCASG, 2022).

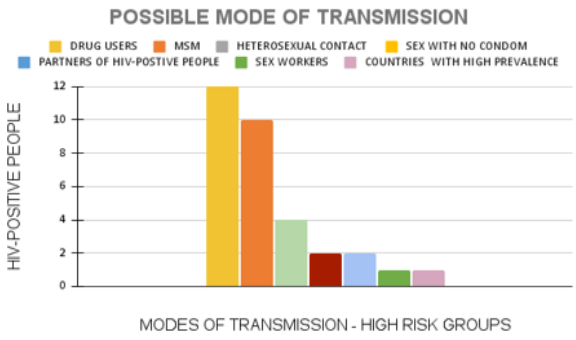

Figure 5. *The possible mode of transmission for HIV-positive people (Data from NRCASG, 2022).*

IV. Conclusions

The reasons for testing newly diagnosed individuals were mostly diagnostic (222 cases), followed by preventive screening (20 cases), detention centers or hospitality structures (16 cases), blood donors (9 cases), and people who inject drugs (8 cases) (Figure 4). The most common modes of transmission were MSM (men who have sex with men) accounting for 31.3%, heterosexual sexual contact (12.5%), unprotected sexual contact (6.3%), partners of HIV-positive individuals (6.3%), and individuals from legal or high-prevalence countries (6.2%) (Figure 5).

The annual Epidemiological Surveillance Report on HIV by the Greek (Hellenic) Centre for Disease Control and Prevention (CDC) for 2021 reported 526 new diagnoses in Greece, with 44.1% attributed to the MSM group and 16.3% to IDUs. By the end of 2021, 19,265 individuals were living with HIV in Greece, with 82.5% being men and 17.3% women. The majority of new diagnoses, over 80%, were in men aged 30-39 (18). Data from the National Reference Center for AIDS of Southern Greece for 2022 align closely with these findings.

In 2022, the GCDC recorded 565 new HIV infections in Greece, with NRCASG confirming 225 new diagnoses, representing 50.9% of new infections. Of these infections, 79.6% were in men and 20.4% in women (18). Similarly, NRCASG reported 288 new diagnoses in 2022, with 78.1% in men and 21.9% in women. MSM accounted for 44.1% of new infections, IDUs for 11.9%, and unprotected heterosexual contact for 21.1% (19, 20).

Across Europe in 2022, 3 million people were living with HIV, with 110,486 new diagnoses. Eastern Europe recorded the highest number of new diagnoses (71.6%), followed by Western Europe (20.3%) and Central Europe (8.1%). The male-to-female ratio was 1.8, with the highest proportion of new diagnoses in the 30-39 age group (36%). Heterosexual sex was the primary mode of transmission (61.2%), followed by sharing contaminated syringes (16.1%) and MSM (11.3%). Mother-to-child transmission accounted for less than one percent of cases.

In summary, the modes of transmission, gender distribution, and age groups most affected by HIV in Europe remained consistent from 2021 to 2022. The increase in new HIV diagnoses in 2022 compared to 2021 highlights ongoing challenges in HIV prevention and control efforts across the continent (3).

V. Acknowledgments

The authors gratefully acknowledge the contributions of the hospitals and clinics: 251 Aviation General Hospital, Labz A to Z Medical Solutions, General Anti-Cancer Oncology Hospital of Athens “Aghios Savvas”, West Attica General Hospital “Aghia Varvara’, University General Hospital Attikon, Eginition Hospital, KAT General Hospital, Thriasio General Hospital of Elefsina, Maternity Hospital Elena Venizelou, Sotiria Thoracic Diseases Hospital of Athens, Children's Hospital Agia Sofia, General Hospital of Samos Agios Panteleimonas, General Hospital of Athens “G. Gennimatas”, “Elpis” General Hospital of Athens, Pammakaristos Hospital of Divine Providence, Ippokrateio General Hospital of Athens, Chalkida General Hospital, Aretaeio Hospital, Therapeutirio of Athens, Leto General Maternity- Gynecological Clinic , Athens Naval Hospital, General Oncologic Hospital of Kifisia “Oi Aghioi Anargyroi”, University General Hospital of Ioannina, Sismanoglio Hospital, Dromokaiteio Psychiatric Hospital of Athens, Psychiatric Hospital of Attica. Their efforts in sending samples to the National AIDS Reference Center of Southern Greece were crucial for the epidemiological surveillance of HIV in 2022.

REFERENCES

1. *Campbell EM, Hope TJ. HIV-1 capsid: the multifaceted key player in HIV-1 infection. Nat Rev Microbiol 2015 Aug;13(8):471-83. doi: 10.1038/nrmicro3503. PMID: 26179359; PMCID: PMC4876022.*
2. *Nuno R. Faria et al., The early spread and epidemic ignition of HIV-1 in human populations. Science 2014;346:56-61, doi:10.1126/science.1256739*
3. *World Health Organization. HIV and AIDS [Internet]. World Health Organization. 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/hiv-aids*
4. *AIDS info | UNAIDS [Internet]. Unaids.org. 2022. Available from: https://aidsinfo.unaids.org/*
5. *ΤΡΟΠΟΙ ΜΕΤΑΔΟΣΗΣ [Internet]. Θετική Φωνή. [cited 2023 May 9]. Available from: https://positive-voice.gr/hiv-aids/transmission*
6. *Prevailing against pandemics by putting people at the centre — World AIDS Day report 2020 [Internet]. Unaids.org. [cited 2023 May 9]. Available from: https://www.unaids.org/en/resources/documents/2020/prevailing-against-pandemics*
7. *WHO. Global health sector strategies on, respectively, HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030 [Internet]. www.who.int. 2022. Available from: https://www.who.int/publications/i/item/9789240053779*
8. *UNAIDS. UNAIDS - KEY POPULATIONS ATLAS [Internet]. kpatlas.unaids.org. 2023. Available from: https://kpatlas.unaids.org/dashboard*
9. *Rodger AJ, Cambiano V, Bruun T, Vernazza P, Collins S, Van Lunzen J, et al. Sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy. JAMA 2016;316(2):171-181.*
10. *Rodger AJ, Cambiano V, Bruun T, Vernazza P, Collins S, Degen O, et al. Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. Lancet 2019;393(10189):2428-2438.*
11. *Με τη συνεργασία της Ελληνικής Εταιρείας Μελέτης και Αντιμετώπισης του AIDS (ΕΕΜΑΑ) ΚΑΤΕΥΘΥΝΤΗΡΙΕΣ*

ΟΔΗΓΙΕΣ ΑΡΧΕΣ ΚΑΙ ΜΕΘΟΔΟΛΟΓΙΑ ΓΙΑ ΤΗΝ ΕΡΓΑΣΤΗΡΙΑΚΗ ΔΙΑΓΝΩΣΗ ΤΗΣ HIV ΛΟΙΜΩΞΗΣ [Internet]. 2020. Available from: https://eody.gov.gr/wp-content/uploads/2020/10/arxes-method-erg-diag-hiv.pdf

12. *Augusto Â do R, Iriemenam NC, Kohatsu L, de Sousa L, Maueia C, Hara C, et al. High level of HIV false positives using EIA-based algorithm in survey: Importance of confirmatory testing. Kumar S, editor. PLOS ONE. 2020 Oct 22;15(10):e0239782.*

13. *Bentsen C, McLaughlin L, Mitchell E, Ferrera C, Liska S, Myers R, et al. Performance evaluation of the Bio-Rad Laboratories GS HIV Combo Ag/Ab EIA, a 4th generation HIV assay for the simultaneous detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum or plasma. Journal of Clinical Virology. 2011 Dec;52:S57–61.*

14. *Alexander TS. Human Immunodeficiency Virus Diagnostic Testing: 30 Years of Evolution. Pasetti MF, editor. Clinical and Vaccine Immunology [Internet]. 2016 Mar 2;23(4):249–53. Available from: https://cvi.asm.org/content/23/4/249*

15. *Montesinos I, Eykmans J, Delforge ML. Evaluation of the Bio-Rad Geenius HIV-1/2 test as a confirmatory assay. Journal of Clinical Virology 2014 Aug;60(4):399–401.*

16. *Parekh BS, Ou CY, Fonjungo PN, Kalou MB, Rottinghaus E, Puren A, Alexander H, Hurlston Cox M, Nkengasong JN. Diagnosis of Human Immunodeficiency Virus Infection. Clin Microbiol Rev. 2018 Nov 28;32(1):e00064-18. doi: 10.1128/CMR.00064-18. PMID: 30487166; PMCID: PMC6302353.*

17. *Wong CC, Lim SH, Tan CT, Lui SY, Lee YL, Chan KP. Performance of the HIV Blot 2.2, INNO-LIA HIV I/II Score, and Geenius HIV 1/2 Confirmatory Assay for use in HIV confirmation. Tse H, editor. PLOS ONE. 2018 Jun 21;13(6):e0199502.*

18. *Εθνικός Οργανισμός Δημόσιας Υγείας, Τμήμα Επιδημιολογικής Επιτήρησης HIV/AIDS. Επιδημιολογική Επιτήρηση του HIV/AIDS στην Ελλάδα Διαγνώσεις έως 31/12/2022 HIV/AIDS Surveillance in Greece Diagnoses through 31/12/2022 ΕΤΗΣΙΑ ΕΚΔΟΣΗ ΤΟΥ ΕΘΝΙΚΟΥ ΟΡΓΑΝΙΣΜΟΥ ΔΗΜΟΣΙΑΣ ΥΓΕΙΑΣ (ΕΟΔΥ) ANNUAL EDITION OF THE NATIONAL PUBLIC HEALTH ORGANIZATION (NPHO) Hellenic Democracy / Ministry of Health [Internet]. 2023. Available from: https://eody.gov.gr/wp-content/uploads/2020/07/epidimiologiko-deltio-hiv-2022-1.pdf*

19. *Resta P., Kotsianopoulou M., Androutsopoulou M., Milaj K., Procter K., Tzanakaki T., Beloukas A. (2022). New HIV Infections Over the Last Year in the National AIDS Reference Center of Southern Greece. 10th Panhellenic AIDS, Hepatitis & Emerging Diseases Meeting.*

20. *Procter K., Resta P, Kotsianopoulou M, Tzanakaki T. & Beloukas A. (2022). Epidemiological Characteristics of New Diagnoses and Transmissible Resistance Surveillance: Data from the National AIDS Reference Center of Southern Greece for 2022. 34th Panhellenic AIDS Conference.*