HIV and Hepatitis C Virus Infection in the United States: Whom and How to Test

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In the United States, of the 1.1 million persons infected with human immunodeficiency virus (HIV) and the 2.7 million infected with hepatitis C virus (HCV), approximately 16% and 50%, respectively, are unaware of their infection. Highly effective treatments have turned both diseases into manageable conditions, and in the case of hepatitis C, a disease that can be cured. Early diagnosis is imperative so that infected persons can take measures to stay healthy, get into care, benefit from therapy, and reduce the risk of transmission. In this report, we review current recommendations provided by the Centers for Disease Control and Prevention (CDC) and the United States Preventive Services Task Force on whom to screen for HIV and HCV infections, and recommendations from the CDC, the Association of Public Health Laboratories, and the Clinical and Laboratory Standards Institute on how to test for these infections.

Keywords. HCV; hepatitis C virus; HIV; nucleic acid test; testing algorithm.

In the United States, approximately 1.1 million persons are infected with human immunodeficiency virus (HIV) [1], and an estimated 50 000 new infections occur each year [2]. The disease burden is highest in men who have sex with men (MSM), in particular, young, black MSM. In comparison, the prevalence of chronic hepatitis C virus (HCV) infection is nearly 3 times that of HIV, with an estimated 2.7 million persons infected with HCV in the US noninstitutionalized civilian population [3], and an estimated 17 000 new infections in 2010. Persons born during 1945–1965 bear the brunt of HCV infection and disease, attributed to injection drug use and/or healthcare exposures, such as unscreened blood transfusions during the 1970s–1980s [4].

HIV primarily infects CD4+ T lymphocytes and causes deterioration of the immune system within weeks to months, followed by deterioration of overall immune function over months to years [5]. HCV infects hepatocytes with slow, and often silent, disease progression over several decades [6]. Both HIV and HCV are blood-borne RNA viruses and are transmitted via contaminated blood (such as from shared injection equipment or needle stick injury) or contaminated blood products. In the United States, sexual transmission among MSM is the primary route for HIV transmission [1]. Although sexual transmission of HCV occurs among HIV-positive MSM [7], injection drug use is the primary risk factor for HCV transmission [8].

Highly potent and effective treatment for HIV has turned HIV disease, once considered a death sentence, into a manageable condition. With early treatment, life expectancies are comparable to those of the general population [9, 10]. The unparalleled advances in HCV treatment within the past few years—from interferon-containing regimens with 40% sustained virologic response (SVR) rates in genotype 1 treatment-naïve individuals [11] to all-oral, direct-acting antiviral regimens
with nearly 100% SVR regardless of the genotype of the infecting HCV [12]—have transformed an illness once thought to be incurable to one for which eradication is possible.

With a significant proportion of infected persons unaware of their infection (approximately 16% for HIV [1] and 50% for HCV [13]), effective treatment provides the impetus for increased screening. Accurate testing and early diagnosis allow infected persons to take measures to stay healthy, get into care, benefit from therapy, and reduce the risk of transmission. This report provides an overview of current recommendations on whom to test and how to test for HIV and HCV in the United States and identifies some remaining challenges.

WHOM TO TEST

Healthcare providers rely on the Centers for Disease Control and Prevention (CDC) and the United States Preventive Services Task Force (USPSTF) for guidance regarding screening for HIV and HCV. The CDC makes evidence-based recommendations relating to the impact of current HIV and HCV screening practices on diagnosis as well as public health outcomes in the United States, including patient populations that may not have been reached with prior testing recommendations. The USPSTF, an independent body of experts in preventive medicine and primary care, reviews evidence for preventive services, including screening, and makes recommendations (graded on a hierarchy of A, B, C, or D or I, for insufficient) based on the strength of evidence and the expected benefit and harm associated with the preventive service. Section 2713 of the Patient Protection and Affordable Care Act requires insurers to cover preventive services with a USPSTF “A” or “B” grade without patient cost-sharing [14]. Alignment of recommendations from the CDC and USPSTF facilitates widespread adoption of HIV and HCV testing recommendations, and improves progress toward national goals outlined in the National HIV/AIDS Strategy [15] and the Viral Hepatitis Action Plan [16].

Human Immunodeficiency Virus

In 2006, the CDC recommended that providers perform routine opt-out screening for HIV for persons aged 13–64 years regardless of risk in all healthcare settings in which the prevalence of undiagnosed HIV is ≥0.1%. This recommendation was supported by a large body of evidence demonstrating the benefits of routine screening, including increased diagnosis of HIV infection, greater patient acceptance when testing is offered to everyone without risk-based targeting, and reduced HIV transmission to partners from infected persons who are aware of their infection [17]. In 2013, the USPSTF upgraded HIV screening for individuals not at increased risk for HIV from a “C” to an “A” grade, based on the benefits of antiretroviral therapy initiation for HIV-positive persons with CD4+ T lymphocyte count <500 cells/µL and its effectiveness for reducing transmission to uninfected partners [18, 19]. This close agreement between the CDC and USPSTF recommendations promotes a concerted, national effort to increase HIV diagnosis and provide opportunities for earlier linkage to care and treatment. The CDC and USPSTF also recommend at least annual retesting for persons at high risk for HIV infection.

Hepatitis C Virus

In 2012, the CDC recommended one-time testing of all persons born during 1945–1965 [4], expanding on previous guidelines [20] that recommended routine HCV testing in persons who ever injected illegal drugs, who received blood transfusions or organ transplants before July 1992 or from a donor later known to be HCV-positive, who were exposed to HCV-positive blood through a healthcare setting, or who were born to HCV-positive women. As persons born during 1945–1965 represent the majority of individuals with rising HCV-associated morbidity and mortality, the 2012 recommendation aimed to increase HCV diagnoses in persons in this cohort who have largely been missed by risk-factor screening. In 2013, USPSTF revised its recommendations from a “C” in the draft recommendation to a “B” grade to offer one-time screening for persons born during 1945–1965. This revision was based on the enlarging evidence base showing associations between achieving SVR and improved clinical outcomes, as well as improved SVR from available therapy [21]. USPSTF also amended its long-standing recommendation from an “I” to a “B” grade to screen persons at high risk of infection.

The CDC and USPSTF recommendations did not offer guidance on how frequently persons at increased risk of infection should receive HCV testing. The latest guidelines from the American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) recommend at least annual testing for persons who inject drugs and HIV-infected MSM, recognizing the high incidence of HCV infection in these subgroups [22].

HOW TO TEST

Human Immunodeficiency Virus

The CDC, the Association of Public Health Laboratories (APHL), and the Clinical and Laboratory Standards Institute (CLSI) constitute the main sources for guidance on laboratory testing for the diagnosis of HIV infection. The CLSI’s Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline is considered the standard of practice by many laboratorians and provides guidance on types of initial and supplement tests, alternative testing algorithms, test result interpretation, and result reporting in special situations, as well as how to establish a quality control
program for HIV testing [23]. For laboratory HIV testing, the CDC and APHL recommend a testing algorithm, herein referred to as the 2014 HIV testing algorithm, that employs, as the initial test, an antigen/antibody (Ag/Ab) immunoassay capable of detecting HIV type 1 (HIV-1) and HIV type 2 (HIV-2) antibodies and HIV-1 p24 antigen (Figure 1). (Commercially available tests are classified into generations depending on the various serologic and virologic markers detected. First- and second-generation tests detect immunoglobulin G (IgG) antibody using viral lysate and recombinant antigens, respectively. Third-generation tests detect IgG and immunoglobulin M (IgM) antibodies, whereas fourth-generation tests detect p24 antigen and IgG and IgM antibodies). If the initial test is reactive, it is followed by an HIV-1/HIV-2 antibody differentiation immunoassay. Positive results on the antibody differentiation immunoassay establish the diagnosis of HIV-1 or HIV-2 [24]. Specimens reactive on the initial Ag/Ab immunoassay that are negative or indeterminate on the antibody differentiation immunoassay are tested with HIV-1 nucleic acid test (NAT), which, if positive, identifies acute HIV-1 infection. Negative NAT results indicate false-positive results from the initial Ag/Ab immunoassay. The 2014 HIV testing algorithm facilitates detection of early HIV-1 infection, produces fewer indeterminate results, and allows more timely identification of HIV-2 infection compared with the previous HIV testing algorithm that relied on HIV-1 Western blot analysis for confirming reactive results [25, 26]. The sequence of subsequent tests in the 2014 HIV testing algorithm will detect additional infections negative by Western blot even if laboratories use a third-generation immunoassay as the initial test. A turnaround time of 1 day is possible for the majority of samples in which antibody is present, but results may be delayed for the small number of samples that need NAT if they require transport to a reference laboratory for testing [24].

One challenge for widespread implementation of the 2014 HIV testing algorithm is the limited number of US Food and Drug Administration (FDA)–approved supplemental tests. Currently, the Multispot HIV-1/HIV-2 Rapid Test is the only FDA-approved HIV-1/HIV-2 differentiation immunoassay that can be used after a reactive Ag/Ab immunoassay result. Moreover, the APTIMA HIV-1 RNA Qualitative Assay is the only NAT that is FDA-approved for the diagnosis of HIV-1 infection. Quantitative NAT (viral load assays) are more widely available than qualitative NAT and could reduce turnaround time for test results, but they are not FDA-approved for HIV diagnosis.

Point-of-care rapid HIV tests are useful for outreach settings to facilitate testing among hard-to-reach populations, for persons who might not return for laboratory test results, and in some circumstances where immediate test results are required for clinical decision making. Sensitivity of rapid

![HIV-1/2 antigen/antibody combination immunoassays](image)

**Figure 1.** The 2014 human immunodeficiency virus (HIV) testing algorithm employs an HIV-1/HIV-2 antigen/antibody immunoassay followed, if reactive, by a supplemental HIV-1/HIV-2 differentiation immunoassay or nucleic acid test for identifying established and acute infections, respectively. Source: Centers for Disease Control and Prevention [24]. Abbreviations: Ag, antigen; HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; NAT, nucleic acid test.
HIV antibody tests for established infection is comparable to that of laboratory assays. However, they become reactive for antibodies later than laboratory-based third- and fourth-generation immunoassays.

After reactive results from any HIV rapid test, including the Alere Determine HIV-1/2 Ag/Ab Combo rapid test, specimens should be tested according to the full 2014 HIV testing algorithm, starting with the laboratory-based Ag/Ab immunoassay. Table 1 provides a list of FDA-approved tests for HIV diagnosis.

Consistent with the changes in diagnostic criteria provided by the 2014 HIV testing algorithm, the CDC and the Council for State and Territorial Epidemiologists revised the surveillance case definition for HIV infection to include infection that occurred within 180 days (including acute HIV infection) classified as stage 0 and specific criteria for defining a case as HIV-2 [27].

### Table 1. Food and Drug Administration–Approved Tests for the Diagnosis of HIV Infection

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
<th>Analytes Detected&lt;sup&gt;a&lt;/sup&gt; (IgG Ab, IgM Ab, p24 Ag, HIV-1 RNA)</th>
<th>Markers Used for Detection&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional enzyme immunoassays</strong></td>
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<tr>
<td>Avioq HIV-1 Microelisa System</td>
<td>Avioq</td>
<td>IgG Ab</td>
<td>Viral lysate, gp160</td>
</tr>
<tr>
<td>Bio-Rad GS HIV-1/2 PLUS O</td>
<td>Bio-Rad Laboratories</td>
<td>IgG and IgM Ab</td>
<td>HIV-1 p24, gp160, group O; HIV-2 gp36</td>
</tr>
<tr>
<td>Bio-Rad GS HIV Combo Ag/Ab EIA</td>
<td>Bio-Rad Laboratories</td>
<td>IgG and IgM Ab p24 Ag</td>
<td>HIV-1 gp41, gp160, group O, p24 monoclonal antibodies; HIV-2 gp36</td>
</tr>
<tr>
<td><strong>Chemiluminescent immunoassays</strong></td>
<td></td>
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<tr>
<td>Abbott Architect HIV Ag/Ab Combo</td>
<td>Abbott Laboratories</td>
<td>IgG and IgM Ab p24 Ag</td>
<td>HIV-1 gp41, group O, p24 monoclonal antibodies; HIV-2 gp36</td>
</tr>
<tr>
<td>Advia Centaur HIV 1/O/2 Enhanced Assay</td>
<td>Siemens</td>
<td>IgG and IgM Ab</td>
<td>HIV-1 gp41/120, p24, group O; HIV-2 gp36</td>
</tr>
<tr>
<td>Ortho Vitros Anti-HIV 1 + 2</td>
<td>Ortho-Clinical Diagnostics</td>
<td>IgG and IgM Ab</td>
<td>HIV-1 gp41, gp41/120, p24; HIV-2 gp36</td>
</tr>
<tr>
<td><strong>Rapid tests</strong></td>
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<tr>
<td>Alere Clearview Complete HIV 1/2</td>
<td>Chembio Diagnostics</td>
<td>IgG Ab</td>
<td>HIV-1 gp41, gp120; HIV-2 gp36</td>
</tr>
<tr>
<td>Alere Determine HIV 1/2 Ag/Ab Combo</td>
<td>Alere</td>
<td>IgG Ab and p24 Ag</td>
<td>HIV-1 gp41, gp120, p24 monoclonal antibodies; HIV-2 gp36</td>
</tr>
<tr>
<td>Chembio DPP HIV 1/2 Assay</td>
<td>Chembio Diagnostics</td>
<td>IgG Ab</td>
<td>HIV-1 gp 41, gp120; HIV-2 gp36</td>
</tr>
<tr>
<td>Chembio HIV 1/2 STAT-PAK</td>
<td>Chembio Diagnostics</td>
<td>IgG Ab</td>
<td>HIV-1 gp41, gp120; HIV-2 gp36</td>
</tr>
<tr>
<td>INSTI HIV-1 Antibody Test K</td>
<td>Biolytical Laboratories</td>
<td>IgG Ab</td>
<td>HIV-1 gp41; HIV-2 gp36</td>
</tr>
<tr>
<td>Multiplex HIV-1/HIV-2 Rapid Test</td>
<td>Bio-Rad Laboratories</td>
<td>IgG Ab</td>
<td>HIV-1 gp41; HIV-2 gp36</td>
</tr>
<tr>
<td>OraQuick Advance Rapid HIV-1/2 Antibody Test</td>
<td>Orasure Technologies</td>
<td>IgG Ab</td>
<td>HIV-1 gp41; HIV-2 gp36</td>
</tr>
<tr>
<td>Reveal G3 Rapid HIV-1 Antibody Test</td>
<td>MedMira</td>
<td>IgG Ab</td>
<td>HIV-1 gp41, gp120</td>
</tr>
<tr>
<td>Uni-Gold Recombigen HIV-1/2</td>
<td>Trinity BioTech</td>
<td>IgG and IgM Ab</td>
<td>HIV-1 gp41, gp120; HIV-2 gp36</td>
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<tr>
<td><strong>Qualitative NAT</strong></td>
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<tr>
<td>APTIMA HIV-1 RNA Qualitative Assay</td>
<td>Hologic Gen-Probe</td>
<td>HIV-1 RNA</td>
<td>TMA; HIV-1 LTR and polymerase genes</td>
</tr>
</tbody>
</table>

Abbreviations: Ab, antibody; Ag, antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IgG, immunoglobulin G; IgM, immunoglobulin M; LTR, long terminal repeat; NAT, nucleic acid test; TMA, transcription-mediated amplification.

* From device package inserts and Food and Drug Administration Summaries of Safety and Effectiveness.

**Hepatitis C Virus**

As there is no CLSI guideline for HCV testing, the testing sequence recommended by the CDC, herein referred to as the 2013 HCV testing algorithm, provides guidance on how to test for HCV infection in clinical practice. Highlights of the algorithm include NAT for antibody-reactive specimens to identify current HCV infection, removal of the Chiron RIBA HCV Strip Immunoblot Assay (RIBA) for supplemental testing, and accommodation of the OraQuick HCV Rapid Antibody test [28] (Figure 2). The algorithm starts with an immunoassay (conducted in the laboratory or using the OraQuick HCV Rapid Antibody Test), which if reactive, is followed by NAT. A nonreactive immunoassay result requires no further testing, except for persons who are immunocompromised or have had recent exposure to HCV and are at risk of having acute infection. A positive NAT result indicates current HCV infection, requiring linkage to care. A negative NAT result, indicating
absence of current infection, requires no further testing in most instances. Repeat NAT is recommended in the following circumstances: recent HCV exposure (within the past 6 months), clinical evidence of disease, or mishandling or improper storage of the specimen. Table 2 provides a list of FDA-approved tests for HCV diagnosis that can be used according to the 2013 HCV testing algorithm.

It is critical to identify currently infected individuals (most of whom are chronically infected) early so steps can be taken to limit disease progression and reduce risks for HCV transmission. In contrast to the 2014 HIV testing algorithm for which HIV rapid tests are not considered to be as sensitive as laboratory-based immunoassays for detecting early infection and thus not incorporated in the algorithm, the OraQuick HCV Rapid

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**Figure 2.** The 2013 hepatitis C virus (HCV) testing algorithm employs an HCV immunoassay followed, if reactive, by nucleic acid testing to identify current infection. Source: Centers for Disease Control and Prevention [28]. For persons who might have been exposed to HCV within the past 6 months, testing for HCV RNA or follow-up testing for HCV antibody is recommended. For persons who are immunocompromised, testing for HCV RNA can be considered; to differentiate past, resolved HCV infection from biologic false positivity for HCV antibody, testing with another HCV antibody assay can be considered. Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.
Antibody Test is comparable in performance to laboratory-based HCV immunoassays and thus can be used for primary HCV screening. Reflex NAT testing on all antibody-reactive tests may be conducted to facilitate faster test result reporting back to the provider and patient, thus allowing for earlier linkage to care and treatment. Although the logistical complexities of specimen storage and retrieval may present challenges to performing reflex NAT, stability studies have indicated that plasma samples can be stored at 4°C, 25°C, or 37°C for up to 7 days without significantly affecting HCV RNA detection [29].

One challenge for the implementation of the 2013 HCV algorithm is the ambiguity that can arise following a reactive antibody test and a negative NAT result, which signifies resolved infection or biological false positivity [28]. An immunoblot-based assay capable of distinguishing between true and false antibody reactivity, when available, might provide further clarity.

The HCV core antigen assay presents an alternative to NAT due to its comparable sensitivity to detect acute infection [30] and its predicted lower costs to run test specimens, as it uses immunoassay reagents and techniques that are less expensive and require less technical expertise and training compared with NAT. Widely accepted in Europe, HCV core antigen testing has potential in the United States to replace HCV NAT.

### Quantitative NAT for HIV and HCV

NATs, currently the only FDA-approved tests capable of detecting viremia, detect (qualitatively) or measure (quantitatively) viral RNA in peripheral blood. Historically, qualitative NATs, sensitive for detecting viral RNA, were initially FDA-approved for screening blood donations, and later for diagnosis. Quantitative NATs (viral load assays) were FDA-approved for disease prognosis and monitoring treatment management. With advances in molecular amplification technologies and the advent of real-time polymerase chain reaction, quantitative NAT now have a very broad linear range and comparable sensitivity to qualitative NAT [31, 32]. Nevertheless, clinical laboratories are still restricted by stipulations in device package inserts and cannot reflexively test antibody-reactive specimens with a quantitative NAT to confirm diagnosis. Because such use is off-label, the Centers for Medicare and Medicaid Services (CMS), which regulates laboratory testing through the Clinical Laboratory Improvement Amendments, requires laboratories to conduct separate validation studies to use quantitative NAT for diagnosis [33]. These validation studies are beyond the capacity of many laboratories to conduct. Moreover, lack of standardized criteria for how to conduct validation studies can result in variation in bench and analytic methodologies across laboratories [34].

Although the FDA regulates device marketing and the CMS regulates laboratory testing, neither body regulates the practice of medicine. Recognizing that quantitative NAT now has a low limit of detection, professional clinical societies advocate using quantitative NAT for confirming the presence of HCV [22] and HIV [35]. This approach permits streamlined and cost-effective diagnosis of current infection with simultaneous determination of viral load prior to initiation of therapy.

### CONCLUSIONS

Highly efficacious treatments for HIV and HCV and the long-term benefits associated with such treatment drive the demand for testing. HIV treatment has transformed a life-threatening...
illness into a chronic condition. Although this condition still requires lifelong treatment, early treatment can improve survival such that life expectancy for a person with HIV infection can be as long as that of uninfected persons [9, 10]. Furthermore, the HPTN 052 study demonstrated that initiation of therapy in infected individuals reduces the risk of HIV transmission by 96%, so treatment is a form of prevention [19]. The US Department of Health and Human Services panel on antiretroviral guidelines for HIV-infected adults and adolescents recommends antiretroviral therapy for all HIV-infected individuals [36] as an important tool to improve health outcomes, increase survival, and prevent new infections.

For HCV, what constitutes the standard of care is changing rapidly with shorter and more tolerable treatment regimens being continuously introduced. Thus, boceprevir and telaprevir, the first-generation protease inhibitors approved by the FDA in 2011, are no longer recommended by AASLD and IDSA for treating the majority of HCV-infected persons [22]. Recently approved sipionexvir and sofosbuvir represent the current standard of care [22] when used with pegylated interferon and/or ribavirin, or with each other in an all-oral regimen, achieving SVR rates reaching 100% [37]. SVR is predictive of lower all-cause mortality, reduced rates of liver-related death, and overall better quality of life outcomes [38]. Current and anticipated all-oral therapy regimens that exclude ribavirin open new possibilities for expanding the number of HCV-infected persons receiving therapy, avoiding significant adverse treatment events, reducing HCV morbidity and mortality, and eliminating HCV. Although the high cost of treatment [39] remains the main barrier to access, the rich pipeline of direct-acting antivirals in phase 2 and 3 clinical trials that are expected to enter the market in 2014–2015 promise more competition that might reduce treatment costs. Modeling studies of HCV treatment scale-up in persons who inject drugs support the potential of HCV treatment as a preventive measure that can dramatically reduce prevalence [40].

The National HIV/AIDS Strategy [15] and the Viral Hepatitis Action Plan [16] provide an overall framework to address the burden of HIV and HCV in the United States by specifying a series of goals: increase access to care, improve health outcomes for infected individuals, and decrease new infections. The CDC and USPSTF recommendations on whom to screen along with the CDC 2014 HIV and 2013 HCV testing algorithms are key to achieving these goals. No matter how efficacious current and coming therapies may be, testing is the critical first step for infected individuals to engage in the healthcare system to receive care and treatment.

Notes

Disclaimer. The views expressed in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention (CDC) or the Department of Health and Human Services.

Use of trade names is for identification only and does not imply endorsement by the Department of Health and Human Services, the Public Health Service, or the CDC.

Financial support. The Forum for Collaborative HIV Research (2013–2014) has received funding from AbbVie, Abbott Molecular, Achillion, Boehringer Ingelheim, Bristol-Myers Squibb, Celera, DDL Diagnostic, Genvetch, Gilead Sciences, GSK, Idexx, Illumina, Janssen, Kaiser Permanente, Medscape, Merck Laboratories, Monogram, Novartis, Pacific Biosciences, PPD, Quintiles, Roche Molecular, Tibora, Vertex, and Viiv Healthcare.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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