Atypical HIV test results when PrEP is prevalent – a need for vigilance in the laboratory

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Since the first diagnostic test for HIV was licensed in 1995, the sensitivity and specificity of these tests has improved dramatically, shortening the time it takes to identify someone as HIV infected. Typically HIV infection leads to a high titre viraemia within 2-3 weeks of infection, and within a few days of onset of the virus becoming detectable, a protein component of the virus core, p24Ag, is usually detectable. The presence of p24Ag stimulates the humoral immune response to begin with the development of both an IgG and IgM response. The IgM response falls after 1-2 weeks, but IgG increases in intensity over many months, until years later there is degradation of the overall immune response associated with progressive HIV related disease.[1] As the HIV-specific immune response matures following initial infection, this can be used to identify individuals as being acutely infected, or having a longer term antibody response. This has been clearly demonstrated using 'Fiebig' staging.[2]

The early use of effective antiretroviral therapy (ART) has impacted on the ability of diagnostic assays to accurately determine infection in early infection. As the maturation and maintenance of an antibody response to HIV requires a sustained antigenic stimulation, the use of ART close to the time of infection can inhibit the immune response by very quickly reducing the amount of virus in the infected cells and bloodstream. This leads to reduced antigenic stimulation, with less antibody production, and less time for any antibodies to mature. This means that the 'typical' diagnostic results seen in infection may differ from the norm. Responses may happen later, not at all, or at levels lower and more fragmented than traditionally seen. In some cases early treatment can lead to seroreversion where, although still infected, there is no detectable antibody response on serum testing. These diagnostic challenges need to be considered when examining specimens from those who have been exposed to or confirmed as having HIV infection, but have also experienced ART as either Post-exposure Prophylaxis (PEP) or early treatment initiation.

In the era of Pre-exposure Prophylaxis (PrEP), these diagnostic challenges are magnified. PrEP involves high-risk individuals taking ART prior to exposure to prevent infection. Any infection will therefore occur in a setting where ART has been consistently, or intermittently, used, potentially altering the exposure of the immune response, and its ability to produce a 'typical' antibody response. There is increasing evidence from a variety of groups, particularly those who test large volumes of blood donors, and from recent data from PrEP trials, that PEP, PrEP and early ART initiation in acute infection, can cause blunting of the HIV-1 antibody response. Both non-reactive

[1] Michael JA et al. The immune response during acute HIV-1 infection: clues for vaccine development. *Nature Rev Immunol* 2010;10:11-23.

[2] Fiebig EW et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003;17:1871-1879.

HIV serology and non-progressive Fiebig profiles have been seen, in a setting where detectable virus in the blood is also unlikely.[3,4]

Identifying breakthrough infections therefore may be a complex task. The British HIV Association have recently published guidelines on the use of HIV PrEP detailing how to manage atypical results (http://www.bhiva.org/PrEP-guidelines.aspx - section 7.5.1). In analysing a patient sample from someone taking PrEP, it will be critical to treat all atypical results as a potential sign of infection and to counsel accordingly. It will take time to determine whether people are infectious with 'atypical' or discrepant antibody profiles, involving multiple tests including Western blot antibody analysis and RNA and proviral DNA molecular assays. Decisions on whether such individuals should remain on PrEP, or receive an additional ART agent to treat their potential infection, are critical for individual management, but may further diminish opportunities to confirm their diagnosis. Incorrect results may also leave people exposed to a potentially less efficacious two drug regimen. Individuals with atypical or discrepant antibody testing while taking PrEP should be advised about the need for repeat testing with combined antigen/antibody assays, or using molecular methods, on cessation of PrEP to ensure they remain HIV negative. It is also important to consider the HIV negative results that these individuals receive. Are these results truly negative, or are they a result of the blunting of the immune response? Therefore any sudden increase in the level of reactivity of the sample in the diagnostic assay, even if it is still below the negative cut-off, should be considered and monitored.

There remains no consensus view on which diagnostic assays will perform better in the era of PrEP. The very small number of reported breakthrough cases (four by March 2018), and incomplete recording of those who have developed any form on 'indeterminate' antibody result, means very few platforms and assays have been assessed.[5,6] Public Health England, in collaboration with colleagues at Imperial College, already investigate individuals who demonstrate unusual immune response to HIV. We will be continuing this work as PrEP IMPACT proceeds, testing specimens from individuals taking PrEP who have atypical results on a range of diagnostic assays. These will cover a range of HIV specific antigens to help determine if there is preferential reactivity against any individual antigen. Such evidence will guide the makeup of diagnostic assays that should be used in PReP monitoring or screening programmes. At AIDS 2018 in July 2018 a session was held discussing this important topic highlighting this is a developing field with much work needed to be done (Strategies for diagnosing and managing acute HIV infection in the context of PrEP and immediate ART - http://programme.aids2018.org/Programme/Session/202),

The PrEP era marks a potential paradigm shift in HIV testing, where for a small population of cases persistent or one-off low level antibody reactivity will require more follow up and consideration. International consensus and experience sharing will be key to highlight the best testing strategies for

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[3] Donnell D et al. The effect of oral preexposure prophylaxis on the progression of HIV-1 seroconversion. *AIDS* 2017 Sep 10;31(14):2007-16.

[4] Fogel JM et al. Brief Report: Impact of Early Antiretroviral Therapy on the Performance of HIV Rapid Tests and HIV Incidence Assays. *J Acquir Immune Defic Syndr* 2017 Aug 1;75(4):426-430.

[5] Thaden JT et al. Seroconversion on PrEP: a protocol for untangling adherence vs. resistance failure. 25th Conference on Retroviruses and Opportunistic Infections (CROI 2018), Boston, abstract 1041, 2018.

[6] Ruone S et al. Brief Report: HIV-1 Evolution in Breakthrough Infections in a Human Trial of Oral Pre-exposure Prophylaxis With Emtricitabine and Tenofovir Disoproxil Fumarate. *J Acquir Immune Defic Syndr* 2016;72(2):129-32

this group of at-risk individuals, and to ensure they have the best information available about what any indeterminate result may mean.

BOX 1

Atypical HIV Results: what to look for

1. Low signals near to cut off in screening assays (either just below or below cut-off)
2. Seroreversion on follow up specimens
3. Discrepant results between assays
4. Slow development of antibody/antigen signal in subsequent samples
5. Weak and/or incomplete banding patterns on Innolia or Western blot

BOX 2

Services at PHE Colindale

1. Wide range of asays (non-standard commercial and in-house ELISAs, proviral DNA, novel sequencing)
2. Western blot to determine antibody specific responses
3. Collation of test results from a variety of platforms to determine assay's sensitive to PrEP
4. Referral to clinic specialising in atypical serologically responses to HIV infection (Difficult diagnoses)