Adapting HIV testing algorithms and clinical advice for people with persistently indeterminate test results - a novel national referral clinical service

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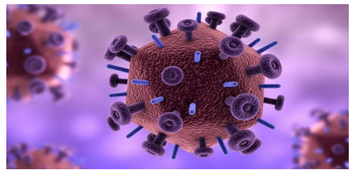
BACKGROUND

- HIV diagnosis relies on detecting HIV-specific antibodies, antigens or viral nucleic acids.
- Certain individuals present with "reactive or indeterminate" HIV results. This usually reflects HIV-specific antibodies at low levels resolved by repeat testing. If reactive test results persist, follow-up normally involves testing for HIV RNA, HIV DNA and additional antibody testing using western blot.
- However, even with these additional tests there are individuals with persistent "indeterminate" test results who remain without a clear HIV diagnosis.
- Such individuals have to live with uncertainty as to their HIV status - their best clinical management is unknown.
- Emerging data indicates that persistent indeterminate HIV test results may become more common with increased use of PrEP, PEP and very early ART initiation, which can delay or alter the development of HIV-specific antibodies.
- **To address this, Public Health England and Imperial College London established a dedicated tertiary referral clinical service to facilitate HIV diagnosis.**

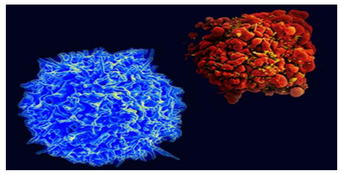
METHODS

- Patients with persistent indeterminate HIV test results consent to be referred to a national referral clinical service for clinical consultation and additional testing and research assays.
- Attendees are invited to donate 60mls of blood divided equally between a national reference centre, clinical hospital, and academic research laboratory for a range of tests (Figures 1-3).



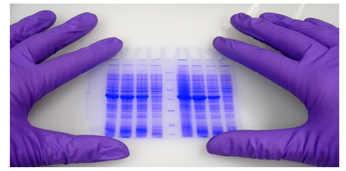
- **Ultrasensitive viral load assay** is able to detect 1 RNA copy/ml making it significantly more sensitive than the standard test.
- **Qualitative whole blood HIV proviral DNA assay**, including integrase resistance assay.
- **Infection and immunology screen are** also performed including HTLV, auto-antibody screen.

Figure 1. Molecular detection of HIV.



- **Routine FACS analysis** quantifies CD4 & CD8 T-cell numbers in peripheral blood.
- **Assessment of immune activation** in response to high degree of viral control.
- **Planned research tests** will quantify HIV-specific CD4 & CD8 responses in peripheral blood, to determine the CD4:8 ratio.

Figure 2. Cellular immune response to HIV.



- **Multiple HIV-1 EIAs** (enzyme immunoassays) performed including commercial assays and in-house typing, to confirm or exclude consistent anti-HIV reactivity across multiple assays.
- **Western Blot** (MP diagnostics) used to determine Fiebig stages of HIV infection, along with likelihood of non-specific EIA results.

Figure 3. Detecting antibody response to HIV.

RESULTS

Table 1. Molecular detection of HIV.

	HIV RNA (copies/ml)		HIV-1 DNA		Presence of HIV virus
	traditional assay	ultra-sensitive	ltr/pol	gag/int	
1	<20	1	-	+	positive
2	<20	0	-	-	negative
3	<20	9	+	-	positive
4	<20	0	-	-	negative
5	<20	17	-	-	positive
6	<20	0	-	-	negative
7	<20	4	+	+	positive
8	<20	5	+	+	positive
9	<20	6	+	-	positive
10	<20	4	-	+	positive
11	<20	0	-	+	positive
12	<20	0	+	-	positive
13	<20	2	-	-	positive
14	<20	0	-	-	negative

Table 2. Measuring CD4 and CD8 levels.

	Cellular immune profile						
	CD4 (300-1400)	CD8 (200-900)	Ratio CD4:8 (1.0-4.0)	CD4 CD25% (15.7-34.9)	CD8 CD25% (4.2-13.6)	CD4 DR% (4.6-10.9)	CD8 DR% (5.7-38.2)
1	807	394	2	19	4	5	13
2	785	315	2.5	26	8	8	23
3	811	415	2	21	6	7	16
4	1359	924	1.5	22	6	8	26
5	1498	518	2.9	12	8	6	18
6	839	401	2.1	26	6	5	17
7	894	872	1	30	12	16	31
8	808	749	1.1	13	5	4	15
9	-	-	-	-	-	-	-
10	1093	588	1.9	38	11	7	42
11	574	330	1.7	30	11	13	32
12	1297	442	2.9	25	5	8	14
13	1129	721	1.6	11	4	4	5
14	751	538	1.4	16	5	5	4

Table 3. Detecting antibody responses to HIV.

	HIV-1 EIA		Western Blot								
	1 S/CO	2 S/CO	p17	p24	p55	p31	p51	p66	gp 41	gp 120	gp 160
1	7.5	4.1	+	+	-	-	-	-	+	-	+
2	15.3	16.8	+	+	-	-	-	-	+	+	+
3	16.5	12.5	+	+	-	-	+	+	+	+	+
4	6.4	18.9	+	-	+	+	+	+	+	+	+
5	11.3	2.5	-	+	-	-	-	-	-	-	+
6	8.1	9.2	-	+	-	-	-	-	-	+	+
7	9.6	3.2	-	+	-	-	-	-	+	+	+
8	9.0	17.6	+	+	-	+	+	+	+	+	+
9	15.8	15.1	-	+	-	+	+	+	+	+	+
10	6.0	17.0	+	+	-	+	+	+	-	+	+
11	12.2	18.5	+	+	+	+	+	+	+	+	+
12	16.4	16.7	-	+	-	-	+	+	+	+	+
13	0.6	1.4	-	-	-	-	-	-	-	-	-
14	0.3	0.1	.	.	.	.	.	.	.	.	.

- USVL RNA results largely corresponds with the detection of proviral DNA on fresh blood draws.
- Despite undetectable RNA by commercial assays, the USVL assay is able to detect RNA down to a single copy.

- Other than the CD4 count for participant 5 and CD8 count for participant 4, all immune cell responses measured are within range.
- No evidence of 'pressure' due to immune control.

- Fresh blood draws has allowed in some cases, better elucidation of reactivity on western blot assay.
- Antibody responses have been integral for confirming or refuting infection in absence of molecular evidence.

CONCLUSIONS

- Using larger blood volumes, freshly processed samples and additional research tests, we have confirmed 11 HIV diagnoses, refuted 1, with 2 unresolved, amongst 14 individuals with persistent indeterminate HIV test results.
- Optimal clinical management for patients with normal immune function and very low, or undetectable, viraemia who are not currently on ART remains uncertain. Patients are invited for annual follow-up.
- One individual on PEP converted to treatment upon diagnosis; one developed high-level viraemia following an intercurrent illness requiring treatment.
- The utility of Western Blot as an essential test for all indeterminate cases has been confirmed.

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