The ability of a peptide-based serotyping assay to differentiate human immunodeficiency virus (HIV) type 1 (HIV-1) subtype B infections from non-subtype B infections was investigated with 166 anti-HIV-1- and HIV RNA-positive (by PCR) serum or plasma specimens. The specimens were divided genetically into those infected with subtype B and non-subtype B by application of a screening heteroduplex mobility assay (HMA) that used plasmids for subtypes A and B alone. Specimens that were not clearly infected with HIV-1 subtype B by HMA or for which the two methods had discordant results in distinguishing those infected with subtype B from those infected with non-subtype B were then investigated with a full HMA plasmid panel and, for selected specimens, env sequencing. For the 141 genotyped and serotypically reactive specimens, the correlation between genotyping and serotyping (all subtypes) was 69%. Of the 67 specimens that reacted monotypically as serotype B, 64 were shown to be infected with genotype B (positive predictive value, 96%). Of the 82 specimens that contained genotype B nucleic acid, 64 reacted monotypically as serotype B (sensitivity, 78%), and 4 specimens reacted with a single non-subtype B peptide; the viruses in 14 specimens could not be assigned a serotype. Initial screening results had indicated that 12 samples had results discordant between restricted HMA and serotyping. The V3 loop amino acids of the infecting HIV strains from the seven specimens with discordant serology results were analyzed. For five specimens discordance occurred when the amino acid sequence of the infecting virus closely resembled those of more than one consensus peptide antigen or when the observed V3 crown motif of the strain was atypical for the genetic subtype present. For the other two specimens no explanation for the discordance was identified. Five specimens gave unclear or discordant results in the initial HMA screen, but the results were resolved when the full plasmid panel was used. Serotyping, although of limited sensitivity, distinguishes between subtype B and non-subtype B infections with a high degree of specificity. However, it poorly differentiates the major non-subtype B subtypes, particularly subtypes A and C. When HIV-1 subtype B predominates, serological typing and/or subtype-restricted HMA screening usefully distinguishes between subtype B and non-subtype B infections.

Phylogenetic analysis of human immunodeficiency virus (HIV) type 1 (HIV-1) strains has revealed two main groups (the M [major] and O [outlier] groups). Group M has been subdivided into at least 10 subtypes (12). Differentiation of these subtypes may be performed directly by genotyping of their gag or env genes or indirectly by characterizing the host’s antibody response to infection by using synthetic subtype-specific peptide antigens derived from the V3 loop of the gp120 glycoprotein of HIV-1 (serotyping) (7, 19). The genetic, antigenic, and biological diversity of HIV-1 may complicate diagnosis, disease monitoring, treatment, and vaccine development (10, 13, 17, 20, 23). This diversity may also be important epidemiologically because it has been suggested that some subtypes may be preferentially transmitted by particular routes (24).

Subtyping of HIV-1 by the detection of subtype-specific antibody in the serum or plasma of infected persons is more convenient and less expensive than the characterization of viral RNA or DNA. Moreover, it is often not possible to recover sufficient RNA from serum or plasma specimens for genotypic analysis. The antigens currently used for serological subtyping of HIV-1 are determined by the V3 region of the outer envelope glycoprotein gp120 (18). Because the antigenically important so-called V3 crown domain differs by only a few amino acids between subtypes, cross-reactivity may occur. The substitution of only one or two amino acids in the V3 crown within a V3 region that is genotypically a particular subtype may result in a V3 crown motif that is identical to that of a heterologous subtype. Differentiation of HIV-1 subtypes by serotyping is thus often complicated by cross-reactive antibodies that suggest an infecting type different from that identified by genotypic analysis.

It may be simpler, in the first instance, to distinguish the common (in Europe and North America) B subtype from the other subtypes, especially because there are distinct and conserved differences between the consensus sequences of subtype B strains and strains of the other subtypes, especially in the V3 crown motif. For subtype B the consensus sequence for the V3 crown motif is GPGR (Gly-Pro-Gly-Arg), while for other subtypes the arginine residue is often replaced by a glutamine (GPGQ) (5).

We report here on the ability of serotyping to differentiate between subtype B and non-subtype B HIV-1 infections as
**CDC PEPTIDES**

Consensus Sequence: KSIHGPQAFFAYT

Group A: D
Group B: D*R
Group C:  
Group D: D*Q
Group E: D*T
Group O: DQEMR*MAWYSGM

**MRC PEPTIDES**

Consensus Sequence: RKSIHGPQAFFAYTG

Group A:  
Group B:  
Group C:  
Group D: R"T
Group E:  
Group F: RKS"L
Group O: IOEMR*MAWYSGM

FIG. 1. Amino acid sequence alignments for the gp120 V3 loop-derived oligopeptide antigens used in the HIV-1 serotyping assays.

*detected genetically by a heteroduplex mobility assay (HMA). Those specimens that were designated as being from patients with HIV-1 subtype B infections by either serotyping or HMA screening but for which the results were not in accord were investigated in greater detail.

**MATERIALS AND METHODS**

*Study population.* Anti-HIV-1-positive serum specimens collected from 121 patients in the course of the Unlinked Anonymous HIV Prevalence Monitoring Programme in England and Wales were examined. These patients comprised 102 patients attending sexually transmitted disease clinics and 19 women receiving antenatal care. A further 45 serum specimens from patients undergoing voluntary confidential HIV testing were included. Only specimens from which a PCR product was available for genotypic analysis were included in the study population of specimens.

*Serotyping.* The peptide-based serotyping assay was performed as described previously (18). Peptide antigens (antigens A to E and O) derived from subtype-specific V3 loop sequences were passively adsorbed onto wells of microtiter plates (Immulon 4; Dynex Ltd., Basingstoke, United Kingdom). 110 μl of a 5-μg/ml peptide solution in carbonate-bicarbonate buffer (pH 9.6) was added to designated wells and the plates were left overnight at 4°C. The plates were then washed once with phosphate-buffered saline (PBS; pH 7.4) containing 0.05% Tween 20 (PBS-T), dried, and stored with desiccant at −30°C. The plates were blocked with 150 μl of milk buffer (PBS containing 0.3% Tween 20 and 5% nonfat dry milk) at 37°C for 1 h and were then washed five times with PBS-T. To each well 100 μl of goat anti-human immunoglobulin G (IgG) peroxidase conjugate (product no. 172-1001; Bio-Rad Ltd., Hemel Hempstead, United Kingdom) diluted 1:4,000 in milk buffer was added, and the mixture was incubated at 37°C for 30 min. The plates were washed five times with PBS-T, and 100 μl of tetramethylbenzidine substrate (product no. 701A; Europa Research, Cambridge, United Kingdom) was added. After 10 min of incubation at room temperature, the reaction was stopped by the addition of 50 μl of 1 M H2SO4. The optical densities at 450 nm were measured. For each specimen, reactivity against each of the peptides was reviewed, and a subtype was assigned to the most reactive peptide on the basis of the following: (i) no other peptide gave an optical density that was >90% of that of the most reactive peptide, and (ii) the OD value of the most reactive peptide was greater than 0.5. Specimens were tested at two dilutions (1/100 and 1/1,000), and, volume permitting, mutually reactive and unreactive specimens were tested at further dilutions to establish a monotypic response. The sequences of the peptides used for screening are shown in Fig. 1. The specimens were also tested by a methodologically identical serotyping assay that used peptides obtained through the UK Medical Research Council (MRC) AIDS Reagent Project.

**Amplification of viral RNA from serum.** RNA was extracted from up to 200 μl of serum by the method of Boom et al. (6), as described previously (11), and converted into cDNA by reverse transcription-PCR (RT-PCR) by modifications of established methods (8, 15). The amplified RNA was recoveried by binding the RNA to silica in the presence of guanidinium thiocyanate, and the RNA was eluted in 40 μl of RNase-free water. Twenty microliters of the eluted RNA was combined with 10 pmol of an HIV-specific antisense primer (primer ED12) (9), denatured for 10 min at 95°C, and immediately cooled on ice. The mixture was incubated at 42°C for 45 to 60 min in 40 μl of a mixture of 50 mM Tris-HCl, 40 mM KCl, 5 mM MgCl2, 0.5% Tween 20, each deoxynucleoside triphosphate at a concentration of 1 mM, 10 mM dithiothreitol, 20 U of RNasin (catalog no. N251a; Promega), and 50 U of Expand Reverse Transcriptase (catalog no. 1785834; Boehringer Mannheim, Lewes, United Kingdom). After stopping the reaction by putting it on ice, the cDNA was immediately amplified by PCR with an Expand High Fidelity PCR kit (catalog no. 1732630; Boehringer Mannheim). An initial denaturation at 95°C for 2 min was followed by 10 cycles of 94°C for 15 s, 50°C for 30 s, and 72°C for 2 min and then 20 similar cycles except for an additional 20-s extension time after each cycle. There was a final 7-min extension at 72°C.

For the second-round PCR, 2 μl of the first-round product was amplified with Expand High Fidelity reagents and the inner primers ED31 and ED32 (9). The thermal cycling conditions for the second-round amplification were the same as those for the first-round amplification except that the annealing temperature was increased to 57°C.

**Genetic determination of HIV-1 subtype.** (i) HMA. *env* gene primers (8) produced 500-bp amplicons encompassing V3 loop sequences. The *env* gene amplicons were subtype by HMA as described previously (3, 16). In brief, HMA was performed with equal amounts of amplificates containing an unknown subtype (100 to 250 ng) and each reference plasmid combined in 50 mM NaCl–5 mM Tris (pH 7.8)–1 mM EDTA, heated to 94°C for 2 min, and incubated at 4°C for 10 min. The specimens were mixed with 1.5 μl of 0.25% bromophenol blue–0.25% xylene cyanol–30% glycerol, and the mixture was loaded onto a 3% MetaPhor XR horizontal agarose gel containing 1 M urea. Electrophoresis was at 125 V for 3 to 4 h in 1× TBE (Tris-borate-EDTA) buffer, and the homoduplexes and heteroduplexes were detected by staining with ethidium bromide. The HMA kit was supplied by the AIDS Reagent Reference Reagent Program, National Institutes of Health, through the MRC AIDS Reagent Project of the United Kingdom (repository reference no. ARP061).

(ii) HMA screening. PCR amplicons from two reference plasmids, subtype A (IC144) and subtype B (SF162), and H2O, as a control, were used for screening. If the faster-migrating heteroduplex corresponded clearly to either of these subtypes, this result was recorded. If no clear result (non-subtype A, non-subtype B) was obtained, the complete panel of reference subtypes (subtypes A to H) was used and the subtype corresponding to the fastest-migrating heteroduplexes was recorded. If the HMA result was still ambiguous or if there was discordance with the serotyping result, the region surrounding the V3 loop was sequenced. (Dis-cordance was defined as a serotype B result when a non-subtype B genotype was identified, or vice versa.)

(iii) Sequencing. *env* gene amplicons were directly sequenced by dye-deoxy terminator reagents with Applied Biosystems reagents and a 373A sequencer (8, 16). Phylogenetic trees of the sequences were constructed with MegAlign (Lasergene; DNASTar) (1), which uses the Chalat and neighbor-joining algorithms. To provide control data, other HIV-positive specimens available to our laboratory and the plasmids from the HMA kit were sequenced by the same methods. Sequences from the Los Alamos HIV sequence database were used as controls in the alignments and phylogenetic analyses.

**RESULTS**

**Differenation into subtype B and non-subtype B.** Of the 166 HIV-positive specimens included in this investigation, the viruses in 122 (73%) were assigned a single subtype by serotyping. A further 19 (11%) specimens reacted with more than one V3 peptide; 25 (15%) were not reactive with any of the V3 peptides used. Of the 141 serotypically reactive specimens, the viruses in 122 were serotyped as HIV-1 subtype B. Genotypic analysis of amplicons from the viruses in 166 specimens identified 82 as having HIV-1 subtype B infections.

Initial testing, i.e., serotyping and HMA screening for subtype B or non-subtype B. Of the 166 HIV-positive specimens included in this investigation, the viruses in 122 (73%) were assigned a single subtype by serotyping. A further 19 (11%) specimens reacted with more than one V3 peptide; 25 (15%) were not reactive with any of the V3 peptides used. Of the 141 serotypically reactive specimens, the viruses in 122 were serotyped as HIV-1 subtype B. Genotypic analysis of amplicons from the viruses in 166 specimens identified 82 as having HIV-1 subtype B infections.
three were monotypically reactive with the HIV-1 subtype B peptide but were genotypically non-subtype B by the HMA screening method (Table 1, specimens 5 to 7). The viruses in the remaining five specimens with discordant results (Table 1, specimens 8 to 12) were classified as non-subtype B by serotyping but as genotype B by the HMA screening method. Sequence analysis confirmed the HMA genotype B result for four specimens but revealed the fifth specimen to be infected with genotype C, as indicated by serotyping. Further testing of this specimen against the full HMA panel also indicated that the virus in that specimen was most similar to genotype C.

Comparison of corrected genotypic and serotypic results. Overall, of the 141 genotyped specimens that were serologically reactive, for only 69% was there an exact concordance between genotyping and serotyping for all subtypes. However, of 70 genotype B specimens that were serotypically reactive, the results for 64 (91%) were concordant. Of 82 specimens that contained genotype B nucleic acid, 64 reacted monotypically as serotype B (sensitivity, 78%). Of 67 specimens that reacted monotypically as serotype B, 40 were concordantly reactive with the MRC peptides (sensitivity, 49%), and of 45 that were infected with genotype B, 40 were concordantly reactive with the MRC peptides (sensitivity, 91%).

Investigation of molecular basis of discordant findings. Sequencing studies with the discordant specimens revealed for four specimens V3 crown motifs atypical to the genotype identified (Table 1, specimens 5 to 7 and 9). In a fifth specimen (specimen 8) a typical subtype B V3 crown sequence was found, but comparison of its entire V3 domain with consensus sequences revealed equal or greater similarity of the domain to subtypes A and F.

Comparison between genotypic and serotypic results with CDC and MRC peptides. Of the 82 serum specimens infected with genotype B, 40 were concordantly reactive with the MRC peptides (sensitivity, 49%), and of 45 that were infected with HIV-1 subtype B as determined by serotyping with the MRC peptides (sensitivity, 91%). Three were monotypically reactive with the HIV-1 subtype B peptide but were genotypically non-subtype B by the HMA screening method (Table 1, specimens 5 to 7). The viruses in the remaining five specimens with discordant results (Table 1, specimens 8 to 12) were classified as non-subtype B by serotyping but as genotype B by the HMA screening method. Sequence analysis confirmed the HMA genotype B result for four specimens but revealed the fifth specimen to be infected with genotype C, as indicated by serotyping. Further testing of this specimen against the full HMA panel also indicated that the virus in that specimen was most similar to genotype C.

### Table 1. Analysis of 12 specimens for which initial genotyping and serotyping results were discordant

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Serotype</th>
<th>Genotype</th>
<th>V3 crown motif</th>
<th>% Similarity of virion V3 peptide sequence to the indicated subtype consensus sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B B Amb</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; A, 60</td>
</tr>
<tr>
<td>2</td>
<td>B B Amb</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 53</td>
</tr>
<tr>
<td>3</td>
<td>B B Amb</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 47</td>
</tr>
<tr>
<td>4</td>
<td>B B Amb</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 47</td>
</tr>
<tr>
<td>5</td>
<td>B B B A</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 53</td>
</tr>
<tr>
<td>6</td>
<td>B B B D</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 53</td>
</tr>
<tr>
<td>7</td>
<td>B B B D</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 53</td>
</tr>
<tr>
<td>8</td>
<td>A ABF Amb</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; D, 53</td>
</tr>
<tr>
<td>9</td>
<td>A BF Amb</td>
<td>B</td>
<td>A</td>
<td>GPGS (non-B) A, 73; B, 73; F; 67</td>
</tr>
<tr>
<td>10</td>
<td>C NR</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; C, 53; F, 60</td>
</tr>
<tr>
<td>11</td>
<td>C B C B</td>
<td>B</td>
<td>A</td>
<td>GPGR (B) B, 67; C, 53; F, 73</td>
</tr>
<tr>
<td>12</td>
<td>C B C B</td>
<td>B</td>
<td>A</td>
<td>GPGQ (non-B) C, 80; A, 73; B, 60</td>
</tr>
</tbody>
</table>

a The HIV-1 subtypes assigned by serotypic and genotypic analyses are shown. For those specimens that were sequenced the amino acid sequence of the V3 crown is also given. Serotyping was by detection of antibodies to panels of peptide antigens supplied by CDC and MRC.

b HMA screening used only plasmids for subtypes A and B.

c NR, nonreactive in the serotyping assay.
d A subtype F peptide was not included in the CDC serotyping assay.
e MR, specimens reactive with more than one peptide.

### Table 2. Correlation of HIV-1 genotyping with detection of antibody against CDC V3 peptides

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>MR</th>
<th>NR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>64</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>14</td>
<td>4</td>
<td>3</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total 16 67 28 4 7 0 19 25 166

a This table takes into account the final result of genotypic screening by HMA with plasmids for subtypes A and B and the further analysis of initially discordant specimens by a full screening of plasmids by HMA or sequencing.
b Serotyping was performed with CDC V3 peptides.
c NR, specimens reactive with more than one peptide.
d NR, nonreactive in the serotyping assay.
e Includes four specimens that initially gave an ambiguous result by the HMA screening method.
f Includes one specimen that gave an initial result by HMA of subtype B.
g Specimen contains subtypes B and E by genotyping.
peptides, 40 were genotype B (positive predictive value, 89%). Concordance between serotyping results obtained with the Centers for Disease Control and Prevention (CDC) and MRC peptides for each of the HIV-1 subtypes from subtypes A to E was 53% (includes reactions with single, multiple, or no peptides). When the MRC peptides were used, 77 specimens were nonreactive or multiply reactive, whereas 44 specimens were nonreactive or multiply reactive when the CDC peptides were used. The results of the serotyping reactions with the MRC peptides for the specimens that gave discordant results between the restricted HMA screening method and serotyping with CDC peptides are shown in Table 1.

DISCUSSION

The accuracy of serotyping with the V3 peptide depends on the infected host’s immune response to an antigen coded for by a very small genetic domain. Even single amino acid substitutions in that domain may significantly affect antigenicity and, therefore, the subtype specificity of the serological response. By contrast, the genetic subtyping methods used in this investigation are dependent on analysis of a much larger domain, in this case over 10 times as large. Consequently, genotypic analysis can be expected to be more specific.

Nonetheless, our results indicate that HIV-1 serotyping based on the CDC peptide antigens can be used with some confidence to discriminate between subtype B and non-subtype B subtypes of HIV-1. This is particularly useful for populations in which subtype B predominates. Precise serological differentiation of non-subtype B subtypes was, however, relatively poor. Although specimens infected with viruses of genotypes C and E frequently reacted specifically with their homologous peptides, specimens infected with viruses of genotype A often reacted most with the subtype C peptide (Table 2). Cross-reactivity between subtypes A and C has been reported before and is probably due to the close similarity of their V3 peptides (22). Although in this study most of the specimens infected with subtype C reacted homologously, the frequent cross-reactions with the subtype C peptide by sera from patients with HIV-1 genotype A infections compromise the predictive value of reactions with the HIV-1 subtype C peptide. Although liquid-phase incubation of blends of peptides with test specimens rather than a simple indirect enzyme immunoassay may improve the effectiveness of serotyping, subtype A and C cross-reactions remain frequent (2).

Other difficulties were the multiply reactive specimens (19 of 166 specimens) whose viruses could not be unambiguously serotyped and the specimens whose viruses were nonreactive with any of the V3 peptides used (25 of 166 specimens). The use of other specimen dilutions or a limited-antigen method (7) might resolve these difficulties. However, lack of a sufficient amount of specimen prevented many of the nonreactive specimens from being tested at lower dilutions. There are several reasons for a lack of reactivity in the serotyping assay, including poor recognition of the V3 loop by the immune system, primary infection in the preceding several months, and compromised immunocompetence.

The advantages of V3 peptide serotyping over HMA are its relatively low cost, its technical simplicity, and the absence of requirements for special and expensive equipment. The cost of HMA-based genotyping may be reduced by using only two reference plasmids, and this also increases the number of specimens that can be processed at any one time; but this approach is most suitable in circumstances in which one subtype (possibly two subtypes) is prevalent. Moreover, our data show that the viruses in specimens may occasionally be misclassified on the basis of their HMA patterns if the whole panel of reference plasmids is not used. The viruses in all four of the specimens that originally gave an ambiguous result by HMA were shown to be subtype B after analysis with the entire plasmid panel, a result that had already been determined by serotyping. The heteroduplexes formed from these specimens ran slowly in the Metaphor XR agarose gel and did not show a classical HMA subtype B pattern, indicating that unless the whole panel is used, correct interpretation of the results may occasionally be made more difficult. The specimen whose virus was wrongly assigned to subtype B by the HMA screening assay (Table 1, specimen 12) ran as the fastest-migrating heteroduplex with the plasmid SF162 (subtype B) used in the screening HMA. It was confirmed to be subtype C only when the virus was sequenced or the specimen was assayed against the full panel of plasmids.

For five specimens whose serotyping and genotyping results were discordant, sequencing revealed that the sequence of the V3 domain was equally similar to more than one subtype-specific peptide or the crown of the V3 loop had a sequence not normally associated with the consensus sequence of that particular genotype. This observation emphasizes how important one or two amino acid substitutions can be to the accuracy of serotypic analysis.

The occurrence of an atypical subtype B GPGR V3 crown motif in the HIV sequences from all three specimens wrongly serotyped as subtype B concurs with the results of Sherafa et al. (21), who suggest that discordant results are due to atypical, nonconsensus V3 sequences in the infecting virus. The viruses in two specimens classified as subtype B by serotyping were shown to cluster with subtype D by sequencing, and it has been reported that in rare cases, as for the two represented here, the GPGR V3 crown motif typical of subtype B may occur in subtype D viruses (5). However, for viruses in two specimens (Table 1, specimens 10 and 11) that were misserotyped, sequencing failed to offer an explanation. Both were only weakly reactive, suggesting that the reactivity detected may have been due to the nonspecific adherence of IgG to the microplate well.

Differences between the CDC and MRC peptides in the efficiency of serotyping may be accounted for by two factors. More importantly, each CDC peptide incorporates a terminal aspartic acid residue whose charge is preferentially attracted to the solid phase of the microplate. This results in more uniform coating and more satisfactory and consistent presentation of the V3 crown to IgG in test specimens. Alternatively, small amino acid sequence differences between subtype-specific peptides from the two sources may sometimes lead to different results.

In conclusion, we have shown that atypical V3 loop sequences can give rise to erroneous results in HIV serotyping assays. Genetic recombination between HIV-1 subtypes might be expected to result in a further reduction of the specificities of serotyping assays (20) and may also result in a reduced effectiveness of genotypic screening methods such as HMA. The limitations of peptide-based serological assays have been described before (14), and our work has identified some of the reasons for this. Serological differentiation of subtype B from non-subtype B infections is mostly adequate and is economical and convenient. For specimens from populations predominantly infected with subtype B, serotyping can be expected to correctly identify the virus in up to 80% of them, permitting targeted use of more expensive and demanding methods for specimens from the remainder of the population.
ACKNOWLEDGMENTS

We are indebted to colleagues from the Hepatitis and Retrovirus Laboratory and the Communicable Disease Surveillance Centre involved in the Unlinked Anonymous Prevalence Monitoring Programme of England and Wales and in the Reference Group of the Hepatitis and Retrovirus Laboratory for allowing access to HIV-positive specimens. We are grateful to P. P. Mortimer for helpful discussions and constructive criticism of this paper. We thank the MRC AIDS Reagent Project for supplying peptides and HMA reagents and the Department of Health of the United Kingdom for funding this project.

REFERENCES