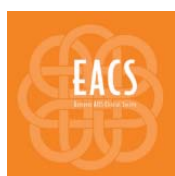


## Panel members

Anne-Mieke **Vandamme**, Belgium; Mounir **Ait-Khaled**, GlaxoSmithKline; Jan **Albert**, Sweden; Birgitta **Åsjö**, Norway; Lee **Bachelor**, Virco; Denes **Banhegyi**, Hungary; Charles **Boucher**, The Netherlands; Françoise **Brun-Vézinet**, France; Ricardo J **Camacho**, Portugal; Bonaventura **Clotet**, Spain; Marie-Pierre **de Béthune**, Tibotec; Andrea **de Luca**, Italy; Stéphane **De Wit**, Belgium; HW **Doerr**, Germany; Stephan **Dressler**, EATG; Rob **Elston**, Roche; José **Gatell**, Spain; Anna Maria **Geretti**, UK; Jan **Gerstoff**, Denmark; William W **Hall**, Ireland; Daria **Hazuda**, Merck; Andrzej **Horban**, Poland; Huldrych **Günthard**, Switzerland; Djordje **Jevtovic**, Serbia; Rolf **Kaiser**, Germany; Max **Lataillade**, BMS; Jens D **Lundgren**, Denmark; Natalia **Marlowe**, Celera; Laura **Maroldo**, Abbott; Michael **Miller**, Gilead Sciences; Claus **Nielsen**, Denmark; Lucia **Palmisano**, Italy; Dimitrios **Paraskevis**, Greece; Carlo Federico **Perno**, Italy; Chris **Petropoulos**, Monogram; Andrew **Phillips**, UK; Mario **Poljak**, Slovenia; Jonathan **Schapiro**, Israel; Jean-Claude **Schmit**, Luxembourg; Rob

**Schuurman**, The Netherlands; Birgitte B **Simen**, 454 Life Sciences; Vincent **Soriano**, Spain; Christoph **Stephan**, Germany; Jukka **Suni**, Finland; Eugenio **Teofilo**, Portugal; Tengiz **Tsertsvadze**, Georgia; Mike **Westby**, Pfizer; Sabine **Yerly**, Switzerland; Mike **Youle**, UK; Anders **Sönnerborg**, Sweden.



In collaboration with the **European AIDS Clinical Society**

# European HIV Drug Resistance Guidelines 2009 update

The European HIV Drug Resistance Guidelines Panel

<http://www.rega.kuleuven.be/cev/>

## CLINICALLY AVAILABLE GENOTYPIC DRUG RESISTANCE INTERPRETATION SYSTEMS

Interpretation System <sup>1</sup>	Source	Levels <sup>2</sup>	Access
HIVdb Version 6.0.1; Stanford, USA (2009-05-06)	Experts Rule-based	S/PL/LL/IR/HR	<a href="http://hivdb.stanford.edu/">http://hivdb.stanford.edu/</a>
Rega V8.0.1, (HIV-1&HIV-2) Leuven, Belgium (2009-02-03)	Experts Rule-based	S//R with drug-GSS weighting factors	<a href="http://www.kuleuven.be/reg/cev/links/reg/algorithm/index.htm">http://www.kuleuven.be/reg/cev/links/reg/algorithm/index.htm</a> <a href="http://hivdb.stanford.edu/pages/asi/">http://hivdb.stanford.edu/pages/asi/</a>
ResRIS (in Spanish); Spanish AIDS Network (2009-01)	Experts Rule-based	S//R	<a href="http://www.retic-ris.net">http://www.retic-ris.net</a>
AntiRetroScan, v2.0; ARCA, Italy (2009-01-10)	Experts Rule-based	100/75/50/25/0 in % activity with drug-GSS weighting factors	<a href="http://www.hivarca.net/includeGenpub/AntiRetroScan.htm">http://www.hivarca.net/includeGenpub/AntiRetroScan.htm</a>
Virco@TYPE HIV-1 report vpt-LM 4.3.01; Virco (2009-01-05)	Database (>59,000 G/P >8,000 TCEs) <sup>3,4</sup>	Quantitative. Lower clinical cut-off at 20% loss of response, upper at 80%.	For information: <a href="http://www.vicolab.com">http://www.vicolab.com</a>
MGRM GeneSeq; Monogram Bioscience (2009)	Experts rules-based and Database (>150 000 G/P)	S/R	For information: <a href="http://www.monogramhiv.com/">http://www.monogramhiv.com/</a>
HIV-GRADE 12/2008; Germany (2008-12)	Experts Rule-based	S//LS/R	<a href="http://www.hiv-grade.de">http://www.hiv-grade.de</a>
Geno2pheno, v. 3.0; Arevir, Germany (2008-12)	Database (>1000 G/P)	S//R Quantitative	<a href="http://www.genafor.org">http://www.genafor.org</a> <a href="http://www.geno2pheno.org/index.php">http://www.geno2pheno.org/index.php</a>
EuResist 1.0 EuResist Network GEIE (2008-09-30)	Database (>3000 TCE plus additional features)	Quantitative. Probability for short-term response with specific drug combinations	<a href="http://www.euresist.org">http://www.euresist.org</a>
ViroSeq™ v2.8, Abbott/Celera (2008-08)	Experts Scores -based	S/P/R	no access or information on line available <a href="http://www.abbottmolecular.com">http://www.abbottmolecular.com</a> <a href="http://www.celera.com">http://www.celera.com</a>
ANRS V17 (HIV-1&HIV-2); France (2008-07)	Experts Rule-based	S//R	<a href="http://www.hivfrenchresistance.org/index.html">http://www.hivfrenchresistance.org/index.html</a> <a href="http://hivdb.stanford.edu/pages/asi/">http://hivdb.stanford.edu/pages/asi/</a>
TRUGENE® GuidelInes™ Rules 14.0; Siemens (Bayer) 2008-06	Experts Rule-based	S//R	no access or information on line available <a href="http://www.labnews.com/">http://www.labnews.com/</a>

1. The here listed version is the one available in September 2009. These algorithms are all clinically evaluated (most retrospectively, some prospectively), and are regularly updated, please visit the indicated websites. 2. S: susceptible; PL: possible low level resistance; LL: low level resistance; IR or I: intermediate resistance; HR: high level resistance; R: resistance; PM: possible multi-NRTI resistance, P: possible resistance, HM: high level of multi-NRTI resistance, H: high level of resistance; LS: low susceptibility; 3. G/P: Genotype/phenotype; 4. TCE: treatment change episodes

Dissemination of these European HIV Drug Resistance Guidelines was supported by unrestricted educational grants from Abbott, Bayer Corporations (now Siemens), Gilead Sciences, GlaxoSmithKline and Hoffman-La Roche

**TABLE 1. RECOMMENDATIONS FOR RESISTANCE TESTING IN THE EUROPEAN SETTING**

<b>Clinical indication</b>	<b>Recommendation to clinicians</b>	<b>Recommendation and evidence level, academic consensus</b>	<b>Motivation</b>	<b>Clarification and Comments</b>
Drug naïve patient (with acute or chronic infection)	Test earliest sample for protease and RT drug resistance. Testing should not delay treatment of acute infection if immediate treatment is being considered. Await resistance test result before starting treatment in chronically infected.	All consensus: 96%	<ul style="list-style-type: none"> <li>Transmitted resistance for protease and RT inhibitors has been observed in most European countries (5-10%)</li> <li>Optimal choice of the first regimen is crucial and should take into account transmitted resistance</li> <li>Expected resistance reversal in absence of drugs supports testing the first sample available</li> <li>When superinfection is suspected (e.g. high risk behaviour), both the earliest and the most recent sample before starting ART may be tested</li> </ul>	<ul style="list-style-type: none"> <li>Most current algorithms do not differentiate their interpretation according to whether the patient is treatment-naïve, however in case there is evidence for transmitted drug resistance, it is suspected that major drug resistance mutations have reverted but are still present as minor variants. In such cases, resistance test results may not reflect the full extent of transmitted resistance and may therefore call for a therapeutic choice taking this 'hidden' resistance into account. Such differential interpretation is still in an exploratory phase.</li> <li>Resistance testing has been shown to be cost effective when levels of transmitted resistance are above 1%.</li> </ul>
Virological failure	Test sample taken on the virologically failing therapy. Test envelope and integrase only when EI resp INSTI were part of the failing regimen.	AI consensus 88%	<ul style="list-style-type: none"> <li>Assessing the contribution of resistance to virological failure allows a better treatment decision, and is also informative whether drugs can be recycled. However, a "regimen sensitive" result for a sample taken on therapy may rise concern regarding adherence.</li> <li>There is some evidence that certain mutations associated with enfuvirtide resistance are also associated with CD4 count rise, despite virologic failure. Clinicians may consider this when deciding whether to stop enfuvirtide as part of a virologically failing regimen.</li> <li>Virologic failure is defined as in <a href="http://www.europeanaidsclinicalsociety.org/">http://www.europeanaidsclinicalsociety.org/</a></li> </ul>	<ul style="list-style-type: none"> <li>Lack of INSTI and EI resistance testing in this case still allows the clinician to install a proper new regimen since no drugs with cross resistant are currently available. However this lack of resistance information impairs the judgement of the clinician on which drugs are actually failing, information which can be useful in the assessment whether there are still other factors associated with this treatment failure.</li> <li>Test results need to be interpreted in view of clinical context, treatment and resistance history</li> </ul>
CCR5 antagonist	In addition to resistance testing at virological failure as mentioned above, for a CCR5 antagonist, test tropism (1) before use as indicated in treatment guidelines, (2) consider testing upon virological failure of a CCR5 antagonist, and (3) consider testing for patients with undetectable viral load for whom a therapy change has to be made and a CCR5 antagonist is considered.	2. CIII consensus:100% 3. CIII consensus:58%	<ul style="list-style-type: none"> <li>A tropism test upon virological failure of a CCR5 antagonist assesses whether a tropism shift was the cause of failure</li> <li>For patients for whom a therapy change at undetectable viral load is needed, tropism testing allows the clinician to assess whether a CCR5 antagonist can be included in the new therapy.</li> </ul>	<ul style="list-style-type: none"> <li>If a tropism test is needed at undetectable viral load, it should be performed on proviral DNA</li> </ul>
Inappropriate treatment interruption of a successful NNRTI containing therapy	Treatment interruption is generally considered not a good strategy, but if needed, appropriate treatment interruption of a successful NNRTI containing therapy is described under the treatment guidelines <a href="http://www.europeanaidsclinicalsociety.org/">http://www.europeanaidsclinicalsociety.org/</a> . At treatment re-initiation and if resistance history is not available, consider retrospective testing a post-stop sample as soon as viral load rises above resistance testing threshold, if such an early sample is available.	CIII consensus 88%	<ul style="list-style-type: none"> <li>In general, at re-initiation after interrupting a successful treatment, if last treatment is resumed, this may be done without resistance testing, except in cases of a combination of drugs with different pharmacokinetics, where accumulation of resistance to the drug with the longest half life can occur during interruption. This recommendation deals with NNRTI's, they have a longer half life than most drugs and a low genetic barrier to resistance and can thus be considered as an exception for which separate guidance is needed.</li> <li>Expected resistance reversal (overgrowth of more susceptible virus) in absence of drugs supports testing the sample within 2 months after treatment interruption</li> </ul>	<ul style="list-style-type: none"> <li>Treatment interruption of an NNRTI containing regimen that failed virologically should be treated as any other virological failure</li> <li>Test results need to be interpreted in view of treatment and resistance history</li> <li>Resistance testing during treatment interruption is difficult to interpret. At treatment re-initiation, it is currently assumed that all archived resistance can re-emerge.</li> <li>If resistance testing is not performed, store the earliest plasma sample for later testing</li> </ul>
Post exposure prophylaxis (PEP)	Use genotypic information from the index case to guide PEP. If this genotype is not known, do not delay PEP, but if a sample from the index case is available, genotype index case to change or simplify PEP if needed.	AIII consensus:92%	<ul style="list-style-type: none"> <li>Resistance test result has to be available as early as possible</li> <li>After 1 week, it is generally believed that changing therapy according to resistance test results is not expected any more to help preventing infection</li> </ul>	<ul style="list-style-type: none"> <li>The most important issue here is timing, hence the recommendation for genotyping.</li> <li>There are no solid scientific data to make a clear timeline of when such resistance data are not useful any more in PEP context.</li> <li>If genotyping is not available, nor from index case nor from transmitted sample, PEP should be based on therapy history of index case, if available</li> </ul>
HIV-2	Consider resistance testing when treatment change is needed after therapy failure	CII consensus:96%	<ul style="list-style-type: none"> <li>Retrospective data indicate the association of some mutations with therapy failure</li> <li>In-house genotypic and phenotypic systems are available in specialized laboratories, if available consider both genotyping and phenotyping</li> </ul>	<ul style="list-style-type: none"> <li>Use of NNRTIs, APV and enfuvirtide is not recommended because of natural resistance</li> <li>Therapy failure should be judged always on CD4 evolution together with viral load</li> <li>Genetic barrier towards resistance is lower than for HIV-1 for some NRTIs and for most PIs. As a result, unboosted PIs are less useful for HIV-2 treatment, and HIV-2 patients are much more difficult to treat than HIV-1 patients.</li> </ul>
<b>Technical issues</b>	<b>Recommendation to laboratory experts</b>	<b>Recommendation and evidence level, academic consensus</b>	<b>Motivation</b>	<b>Clarification and Comments</b>
Which assay to use	1. The panel recommends the use of genotyping in most routine clinical situations. Current genotyping can be performed below a viral load of 1000 copies/ml. 2. Consider additional phenotyping for new drugs, in heavily pretreated patients, and for HIV-2 where genotyping is not easily interpretable.	1. AI consensus:97% 2. CII consensus:100%	<ul style="list-style-type: none"> <li>Genotyping has a more extensive clinical validation, better accessibility, lower cost, faster turn-around time. Consequently additional phenotyping results in a substantial additional cost which can currently only be considered when genotypic guidance is insufficient</li> <li>For new drugs and for HIV-2, there is not sufficient knowledge on resistance mutations.</li> </ul>	<ul style="list-style-type: none"> <li>Some centers can reliably genotype down to a viral load of 300 copies/ml (or sometimes even lower, depending on the protocol)</li> <li>Some genotypic test interpretations are modeling phenotypic information</li> <li>Tropism testing requires separate assay recommendations, however, resistance genotyping can also be used for tropism genotyping</li> </ul>
Interpretation	1. For genotyping, use continuously updated and clinically evaluated resistance interpretation systems and compare the results of different such interpretation systems. Consider discordant interpretations as uncertainty on the resistance profile being scored. 2. Store sequence for future re-interpretation 3. For phenotyping, use clinical cut-off if available, otherwise use biological cut-off. 4. It is recommended to take into account the clinical context, therapy history and resistance history	1. All consensus: 97% 2. AIII consensus: 100% 3. AI consensus: 97% 4. AIII consensus: 100%	<ul style="list-style-type: none"> <li>Both genotypic and phenotypic results can be difficult to interpret, but knowledge is continuously increasing and systems updated and clinically evaluated. Despite these improvements, reliable interpretation systems can still be discordant for particular samples.</li> <li>When interpretation systems are not concordant, the expert needs to make careful statements in his advice towards the clinician, and give more consideration to the more recently updated systems and to systems demonstrating to provide good prediction of treatment response.</li> <li>Resistance reports should be considered as constraints against the use of drugs with evidence of resistance.</li> </ul>	<ul style="list-style-type: none"> <li>A genotypic report for clinicians should include a list of mutations, the interpretation with the system used (algorithm and version), and expert advice.</li> <li>A phenotypic report for clinicians should include information about which test was used (version), fold resistance and cut-off values, and expert advice.</li> <li>Interpretation systems (both genotypic and phenotypic) should be able to discriminate between boosted and non-boosted PIs.</li> <li>Interpretation for divergent subtypes and HIV-2 are currently still more difficult than for HIV-1 subtype B</li> <li>Interpretation of a resistance test results for new drugs requires special attention from an expert</li> </ul>
Laboratory quality control requirements for sequencing proposed to the accreditation authorities	1. Include proper negative and positive controls during extraction/PCR. 2.Editing of the sequence should be traceable. 3.Resistance-related positions should be evaluated by sequencing in 2 directions. 4.Each laboratory should pass at least once a year a proficiency panel test. 5.At least every 2 months or every 50 samples (whatever comes first), a known genotype should be resequenced. 6.Interpretation of the results should be documented.	AIII consensus: 1.97% 2.100% 3.97% 4.100% 5.94% 6.100%	<ul style="list-style-type: none"> <li>Scoring mutations and thus interpretation of resistance test results is influenced by the laboratory performance</li> </ul>	<ul style="list-style-type: none"> <li>Proficiency panel should contain plasma samples with resistance mutations, including with low viral load, different subtypes, samples with mixtures</li> <li>No major discrepancies and &lt;50% minor discrepancies compared to peers using approved tests are required to judge a proficiency panel test result as successful</li> <li>Proficiency panels should only test the performance of laboratories, not the performance of approved tests (which is a research issue, not a lab performance issue)</li> </ul>
Laboratory quality control requirements for phenotyping (RVA) proposed to the accreditation authorities	1. Include proper negative and positive controls during extraction/PCR. 2.Sequence recombinant virus used in the test to ensure proper representativeness of genotypically confirmed resistance mutations. 3.Fold resistance values should be expressed versus a reference laboratory strain which should be included in every run (usually a subtype B) 4.Each laboratory should pass at least once a year a proficiency panel test. 5.Interpretation of the results should be documented.	AIII consensus: 1.100% 2.97% 3.85% 4.100% 5.100%	<ul style="list-style-type: none"> <li>Phenotype and thus interpretation of resistance test results is influenced by the laboratory performance</li> </ul>	<ul style="list-style-type: none"> <li>Proficiency panel should contain plasma samples, including with low viral load, different subtypes, samples with mixtures</li> <li>Compared to peers using an approved test</li> <li>Using a subtype B reference strain assures the comparability of the results. Interpretation may however depend on subtype.</li> </ul>
Storage of sample	If resistance testing can not be performed as indicated, consider storage of recommended plasma (2ml at -80°C)	CIII consensus: 100%	<ul style="list-style-type: none"> <li>In situations where the guidelines for resistance testing have not been followed, retrospective testing might become necessary in the future.</li> </ul>	<ul style="list-style-type: none"> <li>Storage of samples is always a good strategy, including for tropism testing in clinical situations currently not indicated for maraviroc use.</li> </ul>

The recommendations are graded as is usual in guidelines documents for clinical indications: A=recommended, B=strongly consider, C=consider; with indications of the evidence (I = based on at least one prospective randomized study using surrogate markers e.g. viral load, II = based on at least one retrospective study, III = expert opinion based on scientific evidence derived from other clinical and *in vitro* observations); the level of consensus represents only the academic members (expressed as %). APV: amprenavir, EI: entry inhibitor, CCR5: co-receptor for HIV, INSTI: integrase strand transfer inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, PCR: polymerase chain reaction, PEP: post exposure prophylaxis, PI: protease inhibitors, RTI: reverse transcriptase inhibitors.