The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: Still a long way to go

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Abstract Glycated haemoglobin (HbA1c) is considered the ‘gold standard’ for monitoring metabolic control in diabetes. An International Expert Committee recently recommended HbA1c as a better method than measurement of glucose to use in the diagnosis of diabetes, based on its strong association with microvascular complications, a lower day-to-day variability and ease of use, not necessarily in the fasting state. These recommendations have been embraced by the American Diabetes Association (ADA), which stated in its Standards of Medical Care in Diabetes 2010 that “A1c, fasting plasma glucose or the 2 h 75 g oral glucose tolerance test (OGTT) are appropriate for testing diabetes and assessing the risk of future diabetes,” and that “a confirmed A1c ≥ 6.5% is diagnostic for diabetes.” Measuring HbA1c has several advantages over glucose measurements, but its exclusive use should only be considered if the test is conducted under standardised conditions and its limitations are taken into due account.

The impact of its use on the epidemiology of diabetes and other categories of glucose intolerance, as seen from recent reports, is also discussed.

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After Rahbar discovered a diabetic haemoglobin component in people with diabetes in 1968 [1], it was soon demonstrated that this component had chromatographic characteristics resembling those of HbA1c (glycated haemoglobin), which is a minor haemoglobin component [2]. A series of clinical studies then demonstrated a close relationship between HbA1c and mean plasma glucose in the months preceding the test [3,4], while the Diabetes Control and
HbA1c and diagnosis of diabetes

Nowadays, diabetes is an important health problem in the world. The International Diabetes Federation (IDF) Diabetes Atlas has estimated that in 2010, 285 million people around the world have diabetes, representing nearly 7% of the adult world population [11]. It is well known that almost 30% of people with diabetes go undiagnosed [12], and nearly 25% of them have microvascular complications by the time their diabetes is diagnosed [13]. Every effort must consequently be made to diagnose diabetes and the other forms of glucose intolerance (impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)) as promptly as possible [7]. Until now, diabetes has been diagnosed by measuring fasting plasma glucose (FPG) (threshold ≥7 mmol l⁻¹) and/or with the oral glucose tolerance test (OGTT) (≥11 mmol l⁻¹), (see Appendix 1).

In agreement with an International Expert Committee suggestions, the latest ADA Standards of Medical Care in Diabetes 2010 state that ”HbA1c is appropriate for diabetes screening …, a confirmed HbA1c > 6.5% is diagnostic for diabetes,” and that ”mixing different methods to diagnose diabetes should be avoided” [7,14].

Advantages

Several considerations point in favour of the use of HbA1c for the diagnosis of diabetes, the most important of which are listed in Table 1. First, patients must have fasted for at least 8 h before taking OGTT or FPG tests, but this does not apply to HbA1c measurements. The HbA1c test results are also less variable than glucose measurements, the within-individual day-to-day variability being <2% for HbA1c, 12–15% for FPG [15,16] and 16.6% for OGTT [17]. For glucose measurements, we also have to consider errors due to the sample’s treatment prior to its measurement (because even if blood samples are collected in sodium fluoride to avoid glycolysis, their storage at room temperature even for 1–4 h before the measurement is obtained results in a drop in glucose levels ranging from 0.2 to 0.6 mmol l⁻¹ h⁻¹) [18]. In other words, a more dramatic drop in plasma glucose has been reported, on the average of 0.5 mmol l⁻¹ h⁻¹ (range 0.2–1.0 mmol l⁻¹), with stabilisation occurring after approximately 1 h [19]. HbA1c values, on the other hand, are fairly stable after collection [20]. They are also unaffected by short-term lifestyle changes and, because they provide an estimate of the plasma glucose levels over preceding months, they can give a more accurate idea of an individual’s previous glycaemia than FPG or OGTT. Finally, a real advantage stems from using a marker that has been related to the onset of diabetic complications (and microvascular complications in particular) [5,6], and the fact that interventional trials designed to reduce them have focused on HbA1c [5,6,21,22]. HbA1c is consequently familiar to clinicians in this setting because it is currently used to assess prior glycaemic control and to establish therapeutic strategies for diabetic patients.

Limitations

The use of HbA1c has its limitations, as summarised in Table 2, where we have differentiated between the interferences that can in some way be overcome, from the other ones.

As regards the first group, high bilirubin levels may affect some point-of-care testing (POCT) systems, and high triglyceride levels may be a problem in some immunological and glycated haemoglobin assays. The frequency of abnormal haemoglobin disorders varies among different races and different countries; for example, almost 10% of African-Americans have a haemoglobin C trait that could interfere with HbA1c assay [24]. The web site of the National Glycohemoglobin Standardization Program (NGSP, http://www.ngsp.org/interf.asp) provides up-to-date information on how the more common haemoglobin variants (Hbs, Hbc, Hbd and Hbe) interfere with 20 methods most often used to measure HbA1c, and some methods are totally unaffected by the presence of these variants. Of course, if the patient is a homozygous or double heterozygous for a haemoglobin

<table>
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<tr>
<th>Table 1</th>
<th>Advantages in the use of HbA1c for screening and diagnosis of diabetes.</th>
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<tr>
<td>Patient does not need to be fasting</td>
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<td>HbA1c concentration is related to the development of complications</td>
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<td>HbA1c has a smaller intra-individual biological variability (within 2%) respect to that of plasma glucose</td>
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<tr>
<td>HbA1c is not influenced by sudden glycaemic variations (such as under stress) and reflects the mean plasma glucose levels over the last 2–3 months</td>
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<tr>
<td>HbA1c suffers a limited influence from common drugs known to influence glucose metabolism</td>
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<td>HbA1c is already used as an important target for therapy and is familiar to clinicians</td>
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<tr>
<td>The majority of manufacturers of HbA1c kits is already standardized to the current reference systems</td>
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variant, then HbA1c cannot be measured by any method, simply because there is none to measure in the red blood cells (RBCs).

As for the second group, the glycated haemoglobin levels are lower in pregnancy than in women who are not pregnant [25]; hence, the cut-off of 6.5% currently adopted cannot be applied to these subjects. Few data are available as yet on seasonal variations in HbA1c levels, but it seems they may account for a variation of approximately 7%, with 6-monthly cycles [26]. Age can cause an increase in HbA1c levels (inducing an increase of approximately 0.1% HbA1c units per decade from 40 years onwards), as reported by Pani et al., who also found levels 0.4% higher in 70-year-olds than in 40-year-olds with the same glucose tolerance [27]. Race has also been implicated in variations in HbA1c [28,29], the levels in Afro-Caribbeans being 0.4% higher than in Europeans [28], and this can affect diabetes diagnosis [8].

Concerning the third group of conditions listed in Table 2, there are also several factors that interfere with HbA1c measurement to bear in mind. All conditions affecting RBC life and/or turnover, such as iron deficiency anaemia, haemolytic anaemia, chronic malaria, blood transfusion, high blood loss, renal failure or human immunodeficiency virus (HIV) treatment (HbA1c is nearly 10% lower on anti-retroviral therapy), can make the results unreliable [33]. As for iron deficiency, a recent evaluation of the data collected for the National Health and Nutrition Examination Survey (NHANES) study from 1999 to 2006 showed that iron deficiency is common in women, and is associated with a shift in HbA1c to higher levels, mainly from <5.5% to >5.5% [34]. Furthermore, correcting iron-deficient anaemia in type 1 diabetic patients determines a considerable decrease in HbA1c, for the same glucose values, from a mean of 10.1 ± 2.7% before iron treatment to a mean of 8.2 ± 2.7% afterwards [35]. Finally, misdiagnoses are also possible in heavy alcohol drinkers, as alcohol consumption is associated with slightly lower HbA1c concentrations [36].

The unavailability of HbA1c tests in the poorer parts of the world is another obvious drawback to consider, as the currently available point-of-care tests may be useful, but suffer from a number of analytical problems [37]. Most HbA1c assays are significantly less accurate than plasma glucose measurements (i.e., 5.0% vs. 1.0%, expressed as CV) [38], and we have recently emphasised that the method used to assay HbA1c should not exceed the target of 2% for

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<table>
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<th>Table 2</th>
<th>Limitations in the use of HbA1c for screening and diagnosis of diabetes.</th>
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<td>Risk in the diagnosis</td>
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<tr>
<td><strong>Analytical interferences</strong> (could be overcome by appropriate sample handling or by choosing the most appropriate method for HbA1c quantification)</td>
<td></td>
</tr>
<tr>
<td>a) Hyperbilirubinaemia</td>
<td>overdiagnosis</td>
</tr>
<tr>
<td>b) Elevated serum triglycerides</td>
<td>overdiagnosis</td>
</tr>
<tr>
<td>c) Increased WBC</td>
<td>overdiagnosis</td>
</tr>
<tr>
<td>d) Presence of some hemoglobin variants (HbS, HbC, HbD, HbE)</td>
<td>misdiagnosis</td>
</tr>
<tr>
<td><strong>In vivo effects due to physiological conditions</strong> (cannot be handled a-priori)</td>
<td></td>
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<tr>
<td>a) Pregnancy</td>
<td>misdiagnosis</td>
</tr>
<tr>
<td>b) Seasonal variations</td>
<td>over- or mis-diagnosis</td>
</tr>
<tr>
<td>c) Age</td>
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<td>over- or mis-diagnosis</td>
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<td>over- or mis-diagnosis</td>
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<tr>
<td><strong>In vivo effects due to pathological conditions</strong> (cannot be handled a-priori)</td>
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<tr>
<td>a) Type 1 diabetes under rapid development</td>
<td>misdiagnosis</td>
</tr>
<tr>
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<td>misdiagnosis</td>
</tr>
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<td>c) Haemolytic anaemia</td>
<td>misdiagnosis</td>
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<td>e) Recent blood loss, recent transfusion</td>
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<td>f) Splenectomy</td>
<td>overdiagnosis</td>
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<td>overdiagnosis</td>
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<td>h) HIV positive patients on anti-retroviral therapy</td>
<td>overdiagnosis</td>
</tr>
<tr>
<td>i) Erythropoietin and other drugs interacting with erythropoiesis</td>
<td>misdiagnosis</td>
</tr>
<tr>
<td>j) Chronic alcohol abuse</td>
<td>misdiagnosis</td>
</tr>
<tr>
<td><strong>Other reasons</strong></td>
<td></td>
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<tr>
<td>a) HbA1c may not be easily available in some countries</td>
<td></td>
</tr>
<tr>
<td>b) Higher imprecision respect to plasma glucose determination</td>
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long-term inaccuracy [39]. At present, however, the majority of the kits available are unable to achieve this target.

Other factors influencing HbA1c levels and its use

HbA1c and glucose variability

The acute glucose fluctuations occurring in diabetes are considered important in the onset of the chronic complication of this disease. Analysing glucose profiles obtained with continuous interstitial glucose monitoring systems (CGMSs) in subjects enrolled as normal controls in the A1c Derived Average Glucose (ADAG) study, based on fasting plasma glucose, produced some interesting findings [40]. First, 93% of all subjects without diabetes exceeded the IGT threshold of 7.8 mmol l⁻¹, for a median 26 min during the day; 7% of them reached glucose concentrations higher than 11.1 mmol l⁻¹ during CGMS; and the mean HbA1c in these subjects was 5.3 ± 0.3%, that is, within the normal range according to the ADA recommendations [7]. Hence, glucose levels (both FPG and HbA1c values) vary considerably even in people classified as normal [41], casting doubts on the capacity of these parameters to diagnose diabetes correctly. Further studies are therefore needed to establish whether the period of hyperglycaemia in people without diabetes can affect the risk of progression of diabetes.

HbA1c and oxidative modifications

Applying mass spectrometry to the study of HbA1c has provided precious information on the kinetics, entity of its glycation and occurrence of oxidative modifications [42]. It is well known that, by activating oxidative stress, hyperglycaemia plays a pivotal role in the development of chronic diabetes complications. Evidence suggests that not only sustained hyperglycaemia, but also postprandial hyperglycaemia and hyperglycaemic spikes induce superoxide overproduction, giving rise to oxidative stress (glycoxidation products) [43–45].

Using matrix-assisted ionisation mass spectrometry (MALDI), we showed that both α and β globins are glycated to a similar extent [42]. Glyco-oxidation products, as well as glycated products, are present on both globins, but more abundant in diabetic patients than in normal controls. The best linear relationship was observed in the study where abundant in diabetic patients than in normal controls. The as glycated products, are present on both globins, but more to a similar extent [42]. Glyco-oxidation products, as well (MALDI), we showed that both

Cost analysis

The ADA Expert Committee on this topic has yet to report on any cost- and risk—benefit analyses.

The matter can be summed up as follows. In Italy, the National Fee Schedule for the Lombardy Region reports a reimbursement of €12.15 per each HbA1c determination, €1.70 for each glucose test and €2.60 for each OGTT. Judging from these costs, HbA1c is much more expensive than glucose testing. In addition, a complete whole blood and bilirubin count, plus a test such as HPLC to rule out any variant haemoglobins, would be needed to be sure that the HbA1c result is free of the most common pre-analytical interferences. Similar considerations have recently been published by Lippi et al. [47]. Some other factors need to be taken into account, however. First, there are the indirect costs for patients, such as loss of income, which could be estimated at nearly 1 h off work to measure fasting glycaemia and at least 3 h for an OGTT. Second, it is worth bearing in mind that, as the demand for HbA1c testing increases, its cost will probably drop.

HbA1c and other categories of glucose intolerance

The International Expert Committee used retinopathy as the outcome for defining the HbA1c level to be taken into account as diagnostic for diabetes, considering cross-sectional epidemiologic studies on Pima Indians, Egyptians and NHANES populations. In these studies, it was shown that the prevalence of retinopathy was increasing substantially with HbA1c values rising from 6% to 7% [48]. Moreover, the recent analysis drawn from the Evaluation of screening and early detection strategies for type 2 diabetes and impaired glucose tolerance (DETECT-2) trial showed, using receiver operating characteristics (ROC) curve analysis, that the optimal cut-off for detecting retinopathy was an HbA1c of 6.5% [14]. The relationship between HbA1c and macroangiopathy, a very well known and worrying complication of diabetes, is less clear. While some studies found no relationship between these two variables, and others found a capacity to predict cardiovascular disease only at values ≥6.5%, and only in women [49,50], a few prospective studies have described a positive, continuous relationship between HbA1c levels and cardiovascular disease [51]; in particular, a recent study by Selvin et al., with a 6–16-year follow-up of non-diabetic individuals, found HbA1c associated with the diagnosis of diabetes in the same way as FPG, and associated even more strongly with the risk of cardiovascular disease and death from any cause [52]. It should be emphasised, however, that HbA1c was measured in whole blood samples that had been frozen for more than 10 years and using a DCCT-standardised assay, while traceability to the DCCT only applies to fresh blood samples [53]. Other studies are needed to confirm this relationship, moreover, because risk factors other than glucose are involved in this diabetic complication, as also shown by a recent post hoc analysis of the Action to Control in Diabetes (ACCORD) trial [54].

The International Expert Committee, considering that, as for glucose measurement also for HbA1c levels, a continuum of risk for the development of diabetes has been demonstrated [55], identifies people with HbA1c levels between 6.0% and 6.4% as being at high risk of diabetes and recommends that
these people should receive clear effective preventive interventions; but there is no information about the metabolic characteristics of this high-risk class. The only report from Lorenzo et al. [56] involving 855 subjects participating in the Insulin Resistance Atherosclerosis Study, showed that HbA1c is less sensitive in detecting individuals at risk of diabetes than FPG or OGTT, and that it has a lower correlation with insulin resistance and secretion than FPG or 2-h PG. The possibility that the consistency between PG and HbA1c is the same as the level of agreement between FPG and 2-h PG, as demonstrated by the Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe (DECODE) study, also needs to be confirmed [57].

Furthermore, a low-risk class has been added, that is, below the threshold of 6.0%.

However, what is the real risk of diabetes developing later on in these two new risk populations? Indeed, recent studies have reported a greater risk of diabetes developing at lower glycaemic levels than those considered normal [58,59]. A recent systematic review of seven studies examining incident diabetes across a range of HbA1c levels between 5% and 6.5% showed that HbA1c levels between 6.0% and 6.5% are associated with a 25–50% of diabetes over 5 years, while levels between 5.5% and 6.0% are associated with a 9–25% incidence and levels between 5.0% and 5.5% with an incidence of <9% [60]. The review did not compare potential differences in the incidence of diabetes associated with different glycaemia tests (i.e., HbA1c, FPG and OGTT); hence, further studies will be necessary to establish whether HbA1c is more suitable for predicting diabetes than the other tests currently used.

**HbA1c and diagnosis of diabetes: epidemiological studies**

A number of studies have recently been published on this issue and some of them warrant consideration (Table 3, Appendix 2). Judging from the above-mentioned studies, it seems that using HbA1c alone to diagnose diabetes would lead to a number of diagnoses being overlooked, while the sensitivity and specificity of the assay could be improved by combining it with the OGTT.

**HbA1c standardisation**

The effort to standardise the HbA1c test dates back at least 30 years. The most recent and comprehensive approach is the one promoted by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) that...
addressed this issue in 1995 by creating a working group committed to this topic (IFCC WGHbA1c). Thanks to the activities of the WGHbA1c, a reference measurement procedure is now available [70], two primary reference materials have been produced [71], an international network of reference laboratories for HbA1c measurements has been created [72] and master equations have been developed to convert the findings obtained with the IFCC reference system into results aligned with the system of the NGSP [73].

The standardised measurement of HbA1c is now a well-defined matter, agreed on a worldwide basis since the consensus document was signed in 2007 by officers from the ADA, the European Association for the Study of Diabetes (EASD), the IDF and the IFCC [74]. This document, like the one that followed (published by representatives of the IFCC and the companies that manufacture this test) [75], states that all manufacturers of HbA1c diagnostic tests should have been aligned to the IFCC reference system by 31 December 2009, at the latest.

What now remains to be accomplished is the deployment of this reference system at the end-user level (at public health laboratories and diabetes treatment centres, amongst general practitioners and patients). Different actions have been taken in different countries, and no comprehensive report on this topic is available as yet. A working group was created in Italy for this purpose, and it has issued some recommendations [39], the most important of which are listed in Table 4.

In the near future, a great effort will be made to promote an information campaign intended for all stakeholders (medical laboratories, general practitioners, diabetologists and diabetic patients and all medical specialists involved in managing diabetic patients) to ensure a synchronous and smooth transition to the new IFCC reference system throughout the country. Various indicators will be used in the long term to ensure that all (or at least most) laboratory professionals use the IFCC standardised method within the target margins of error. Laboratory professionals should ask the manufacturers of diagnostic kits for evidence of their alignment to the IFCC reference system to ensure consistency with the reference system and to establish the related uncertainty right from the production process onwards.

Table 4 Recommendations for the implementation of international standardization of HbA1c in Italy.

<table>
<thead>
<tr>
<th>Topic</th>
<th>Recommendation</th>
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<tr>
<td>Analytical error limits laboratory practice</td>
<td>The goal of the total allowable error on a single HbA1c measurement should not exceed ± 6.7% (as a percentage fraction of the absolute HbA1c value). The long-term imprecision of the method should be no more than 2%. An adequate internal quality program has to be used to ensure that this imprecision goal be met by the HbA1c method. Participating in EQAS programs, in which commutable materials are used, and adopting HbA1c values assigned using the IFCC reference measurement procedure, are the best way to establish whether measurements that are obtained meet the standards for total error stated above.</td>
</tr>
<tr>
<td>Calibration Reporting units</td>
<td>HbA1c should be measured using methods calibrated to the IFCC reference system. The result should be reported in mmol/mol and % units derived using the master conversion equation [43].</td>
</tr>
<tr>
<td>Timing</td>
<td>Beginning 01 January 2010, HbA1c results will be expressed in units aligned with the DCCT system (%) and in standardized IFCC units (mmol/mol). Next, beginning on 01 January 2012, HbA1c results will be expressed in IFCC units (mmol/mol) only.</td>
</tr>
</tbody>
</table>

Conclusions

Looking back at the balance between the pros and cons of using HbA1c in the diagnosis and screening of diabetes, the weaknesses seem to outweigh the benefits. For the time being, the lack of a cost–effect analysis, the limited availability of the assay in the less developed countries, the epidemiological studies showing small numbers of people being diagnosed with diabetes when this method was used, and racial variability, make it difficult to use HbA1c to diagnose diabetes all over the world. Moreover, evidence of the standardisation of the HbA1c assay may be clear in some countries, but not in others, wherein independent quality audits are not mandatory.

We would therefore discourage the use of HbA1c for diagnosing diabetes for the time being, particularly with the proposed cut-off of 6.5%, unless co-morbidities can be ruled out, the person is under 70-years-old and the method is IFCC standardised and/or NGSP aligned. The HbA1c test should not be used to diagnose diabetes in pregnancy.

We are nonetheless convinced that using HbA1c to rule out diabetes in subjects at risk of developing the disease could significantly reduce the number of other tests (e.g., OGTT) performed and result in a better diagnostic sensitivity and specificity (providing the assay is standardised). It is also worth noting that HbA1c can be higher in ‘normal’ individuals (FPG < 126 mg dl⁻¹ and 2 h OGTT < 140 mg dl⁻¹) with an altered daily glucose profile (41), suggesting that it might be an earlier marker of dysglycaemia than FPG or OGTT.

Another relevant point, in our opinion, remains the weakness of adopting more than one diagnostic criterion to diagnose diabetes. It would be advisable to use just one test to diagnose diabetes the world over because this would enable a better comparison of epidemiological data; it would seem unfeasible, however, because diabetes is a very heterogeneous disease. The only recommendation that can feasibly be made is therefore that patients be tested for diabetes using only one test (i.e., FPG), and that the results be confirmed using the same test at any future visits to avoid the difficulty of interpreting different test results.
Acknowledgement

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Appendix

Supplementary material

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.numecd.2011.02.006.

References


