EMQN External Quality Assessment scheme for Hereditary Recurrent Fevers (2009)
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Dear Colleague,

Thank you for participating in the EMQN EQA scheme for HRF testing. The reports from all EMQN schemes have been marked in the course of a general meeting of all scheme providers and assessors on February 22nd-23rd in Budapest, Hungary. Using this approach, we are working towards applying consistent marking criteria with harmonisation across all our schemes. This document is the final overall scheme report - your individual report is also available from your website account. The expected results and assessment criteria are set out below. Please read all sections of the report carefully, and consider whether anything in your current practice should be changed on the basis of this report.

APPEALS PROCEDURE
If you feel that we have made any mistakes in the assessment of your returns you may submit an appeal to re-evaluate your report assessment. To submit an appeal, please log into your EMQN account. On the web page for this scheme you will find the appeals submission activity - download a copy of the appeals form from there. When you have completed it, upload it back to the web page (see p11 of the EMQN website user guide – copy available from the scheme page). You have until the 14th April 2010 to reply to EMQN. Thereafter the marks become final. You will receive copies of tables showing the final genotyping and interpretation scores for all participants in all the schemes after completion of the appeals procedure. Please remember that the aim of this quality assessment is to be educational, not punitive, and that we are trying to assist laboratories in their continuous efforts towards higher quality of service.

PARTICIPANTS
49 laboratories from 22 countries had registered for the scheme – 47 returned reports. The participating countries are listed below.

Breakdown by country

![Bar chart showing participants by country]
STRUCTURE OF THE SCHEME

This scheme was designed to assess laboratories’ abilities to correctly detect possible mutations in the MEFV, MVK, TNFRSF1A and NLRP3 genes and to interpret these findings. The DNA samples that were distributed had been prepared from peripheral blood samples and their genotypes had been validated on the same batch of DNA independently in 2 laboratories. Diagnostic requests for the (mock) clinical cases were sent together with the samples. Prior to distribution, the laboratories were requested to indicate which genes their centre offered testing for to receive appropriate samples for the scheme.

EVALUATION CRITERIA OF THE REPORTS

CASES

Case 1 (MEFV): Fatima HAYANI (dob 27/04/2000), from Morocco, developed short episodes (1-2 days) of recurrent fever associated with polyarthralgias, erytematous skin rash on both legs and abdominal pain. These episodes started at 5 years of age and recurred every 3-4 weeks. There is a family history of the same disease in her younger brother. She is now successfully treated with oral colchicine. Her Paediatrician suggested a clinical diagnosis of Familial Mediterranean Fever. Please, restrict your analysis to the MEFV gene and report your result.

Case 2 (MVK): Anne HACKETT (dob 12/08/1965), from Ipswich in the United Kingdom, is clinically asymptomatic. She is the mother of Michael (dob 26/05/1998), who has suffered since he was 6 months old from episodes of recurrent fever and adenopathies. These recur every 5-6 weeks. There is no familial history of the disease. Anne reports that her son was diagnosed as suffering from Hyper-IgD and periodic fever syndrome (HIDS) after a positive MVK gene mutational analysis was obtained. The Geneticist suggests performing MVK mutational analysis in Michael’s parents as a part of the genetic counselling process. Please, restrict your analysis in Anne to the MVK gene and report your result.

Case 3 (TNFRSF1A): Carlos PÉREZ (dob 07/11/1960), from Santiago de Chile, suffers from long inflammatory episodes with fever associated with myalgias, migratory erytematous skin rash at chest, arms and legs, conjuntivitis and abdominal pain. These episodes started at 2 years of age and he has had 4-6 episodes every year. Recently, a chronic renal failure has been detected. Carlos has two healthy daughters (15 and 13 years old) but his late mother also suffered from recurrent fever and renal failure. Her Physician suggested the possibility of a TNF receptor associated periodic syndrome (TRAPS) diagnosis. Please, restrict your analysis to the TRAPS-associated gene and report your result.

Case 4 (NLRP3): Sandra PELLEGRINI (dob 07/07/2005), from Pavía in Italy, suffers from an early severe inflammatory disease characterized by fever, recurrent arthritis, generalized urticarial skin rash, severe headache, papilledema, endocraneal hypertension, seizures and frontal bossing. There is no familial history of the disease. Her Paediatric Rheumatologist suggested the possibility of a CINCA/NOMID syndrome and suggested to Sandra’s parents that NLRP3 gene mutational analysis should be performed. Her parents agreed to this. Please restrict your analysis to the NLRP3 gene and report your result.

GENOTYPING

(1) The full score for genotyping was 2.00 marks for each case.

Expected genotype results:

<table>
<thead>
<tr>
<th>Case</th>
<th>Name</th>
<th>Sex</th>
<th>Date of Birth</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fatima HAYANI</td>
<td>FEMALE</td>
<td>27/04/2000</td>
<td>MEFVgene: heterozygous for c.2177T&gt;C (p.Val726Ala); c.2282 G&gt;A (p.Arg761His) and c.442G&gt;C (p.Glu148Gln).</td>
</tr>
</tbody>
</table>

Mutation nomenclature according to Genbank accession numbers; MEFV NM_000243.1; MVK NM_000431.1; TNFRSF1A NM_001065.2; NLRP3 NM_004895.3

INTERPRETATION AND REPORTING:

For this year written report, the interpretation, reporting format and style were not marked.
Points not leading to deduction of marks if absent this year:

1. Date of referral/arrival noted
2. Laboratory reference noted
3. Reason for referral restated
4. Individual reports issued
5. Report clear and concise
6. Report dated
7. Signed by two suitably qualified persons
8. Referring clinician correctly identified.
9. Patient name correctly spelled throughout the report
10. DOB present and correct
11. Patient ID present and correct

According to these criteria, the following results were expected for the cases:

<table>
<thead>
<tr>
<th>Name</th>
<th>Criteria</th>
<th>Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotyping</td>
<td>Correct genotype: all mutations found at both nt and protein levels (including those debated) in relation with the extent of the screening strategy. Failure to provide the correct genotype at the protein level: 1 mark was deducted per missing mutation in recessive diseases, and 2 in dominant diseases. This year, we did not deduct points for failure to provide the correct (cDNA) name of a mutation although this is important for a diagnostic laboratory, but 0.5 mark will be deducted starting from next scheme. Sometimes, genotypes were mis-positioned or mis-called. We deducted 0.5 if typographic mistake; and 1 if misunderstanding. When two names (i.e. usual and gold standard) are recorded for a single mutation (e.g. C30R and C59R for TRAPS) we only commented this year, but we will deduct 0.25 if both names are not given when applicable in the next schemes.</td>
<td>2.00</td>
</tr>
</tbody>
</table>
| Interpretation| Reports meets pre-assigned points:  
CASE 1: V726A and R761H confirm diagnosis if each mutation is on a different parental chromosome (genetic counseling). E148Q clinical significance debated. Existence of a complex allele. The possibility that the mutations could be in cis or in trans should have been discussed.  
CASE 2: Carrier state confirmed. This was an asymptomatic individual, the mother of a patient. Two mutations were not expected.  
CASE 3 and CASE 4: Typical dominant mutations. Diagnosis confirmed (genetic counseling).  
Missing interpretation points: e.g. for mutation E148Q and P369S (MEFV); for mutation P46L and R92Q (TNFRS1A) for mutation V198M (NLRP3): failure to mention "clinical significance is still under debate, or these are susceptibility alleles for inflammation".  
Wrong interpretation (e.g. diagnosis confirmed when only one mutation or failure to mention the possibility of complex alleles if >two mutations not phased are found in recessive diseases).  
Novel missense mutation failure to mention "clinical significance is unknown".  
If counseling is relevant but not mentioned in report (e.g. transmitting parent or de novo mutation for dominant diseases, and phase for recessive disease). | comment |

Comments
OVERALL RESULTS
A total of 49 labs had registered for the scheme and 47 participants returned reports. The distribution of genes that the different centres tested for are shown below:

![Gene distribution chart]

GENERAL COMMENTS ON GENOTYPING
Forty seven out of 49 achieved full marks for the MEFV gene. Three laboratories failed to provide the reference sequence. Most laboratories failed to refer to HGVS nomenclature

Genotyping error rates:

<table>
<thead>
<tr>
<th></th>
<th>No. of cases completed</th>
<th>Average score</th>
<th>No. of errors</th>
<th>Error rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>45</td>
<td>1.81</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Case 2</td>
<td>21</td>
<td>2.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 3</td>
<td>26</td>
<td>2.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 4</td>
<td>22</td>
<td>2.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>1.95</td>
<td>1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

METHODS
There is no consensual or suggested method. However, both method and extent of screening should be provided. This was commented this year, but 0.5 marks will be deducted since next scheme.

INTERPRETATION
*Interpretation was not marked this year, but commented only. Assessors noted that the most frequent comment related to genetic counselling:*

Genetic counselling is recommended in most cases because rules, costs, and habits may differ across countries

- Recessive disease: for phasing and/or for phenotype genotype correlation, and the risk for the rest of the family members.
- Dominant disease: to assess the parental origin of the mutation, the de novo occurrence of the mutation, and or for phenotype genotype correlation, and the risk for the rest of the family members.
REPORTING
The best reports were concise and provided a short and clear answer to the specific clinical question. It is good to keep the report restricted to a single page (without resorting to very small font sizes). It is not necessary and may even be counterproductive to provide extensive background information on HRF. This would be adequate for a genetic counselling letter but is not usually helpful in a diagnostic report. The reports should not contain non pathogenic variants (e.g. R202Q, MEFV; S52N, MVK; Q703K, NLRP3; and all conservative SNPs) and row data. There is a suggestion of report models with minimal content at the end of this report.

PROBLEMS WITH THE SCHEME
After the scheme samples had been shipped out to participants, the organiser was contacted about a problem with 3 of the samples for this year scheme (cases 2, 3 and 4). The batch numbers on the samples did not match that on the scheme documentation (which also included a scanned image of the samples labels). A full root cause investigation of the problem was carried out immediately and identified the cause as a problem with the mail merge process that is used to generate the labels. The labs in question who received the wrongly labelled samples were contacted immediately to ask to destroy the samples they had received and wait for a new batch of correctly labelled samples to be shipped before starting their analysis. The organisers would like to apologise again for the error and assure the participants that we did take this mistake very seriously and have tightened up the QC checks significantly for the following year.

FINAL COMMENTS
This was the first time the HRF scheme was offered by EMQN and the scheme will be available again next year.

Again, we wish to thank all participants and the EMQN staff for their hard work, prompt returns and their co-operation during this exercise. We hope you found it useful to take part. We look forward to your participation later this year which will again be announced by the EMQN office in Manchester. Registration will be through the EMQN web site as before.

With our best wishes,
Yours

Ivona Aksentijevich, Juan Arostequi, Yael Shinar and Isabelle Touitou,
EXAMPLE REPORTS

LABORATORY identifiers
(EMQN lab number)

GENETIC DIAGNOSIS OF FMF
(Familial Mediterranean fever)

Subject: Fatima HAYANI
Date of birth: 27/04/2000
Patient ID: EMQN case (1) or Sample ID: EMQN number (09001896) or internal ID
Type of sample: DNA

Method: Method (sequencing, kit etc…) and extent of the screening (exon 10 or 2 and 10 or…) of the MEFV gene (ref seq: NM_000243.1). This allows identification of XX% of the known mutations.

Results:
Mutations (note: E148Q expected only if exon 2 screened):
1. p.Glu148Gln c.442G>C The clinical significance of this variant is still under debate
2. p.Val726Ala c.2177T>C
3. p.Arg761His c.2282 G>A

Genotype: p.[Glu148Gln(+)Val726Ala(+)Arg761His] Presence of a complex allele

Interpretation:
At least two pathogenic mutations (p.Val726Ala and p.Arg761His) have been identified.
The diagnosis of FMF is highly probable

Genetic counseling is advised to resolve the parental origin of the mutations.

Date of report
Name of the biologist(s)
GENETIC DIAGNOSIS OF MKD
(mevalonate kinase deficiency)

Subject: Anne HACKETT
Date of birth: 12/08/1965
Patient ID: EMQN case (2) or Sample ID: EMQN number (09001899) or internal ID
Type of sample: DNA

Method: Method (sequencing, kit etc…) and extent of the screening (exon …) of the MVK gene (ref seq: NM_000431.1). This allows identification of XX% of the known mutations.

Results:
Mutation: p.Val377Ile c.1129G>A
Genotype: p.[Val377Ile]+[=]

Interpretation:
One pathogenic mutation has been identified. The carrier status of Anne Hackett is confirmed

Date of report
Name of the biologist(s)
GENETIC DIAGNOSIS OF TRAPS
(TNF receptor associated periodic syndrome)

Subject: Carlos PÉREZ
Date of birth: 7/11/1960
Patient ID: EMQN case (3) or Sample ID: EMQN number (09001901) or internal ID
Type of sample: DNA

Method: Method (sequencing, kit etc…) and extent of the screening (exon 1-3-4 or …) of the TNFRSF1A gene
(ref seq: NM_001065.2). This allows identification of XX% of the known mutations.

Results:
Mutation: p.Cys59Arg c.175T>C This mutation is also known as C30R
Genotype: p.[ Cys59Arg]+[=]

Interpretation:
One pathogenic mutation has been identified. The diagnosis of TRAPS is confirmed
Genetic counseling is advised to resolve the parental origin of this mutation.

Date of report
Name of the biologist(s)
GENETIC DIAGNOSIS OF CAPS
(Cryopyrin associated periodic syndrome)

Subject: Sandra PELLEGRINI
Date of birth: 7/7/2005
Patient ID: EMQN case (4) or Sample ID: EMQN number (09001897) or internal ID
Type of sample: DNA

Method: Method (sequencing, kit etc…) and extent of the screening (exon 3 or…) of the NLRP3 gene (ref seq: NM_004895.3). This allows identification of XX% of the known mutations.

Results:
Mutation: p.Asp303Asn  c.907G>A  This mutation is sometimes called D305N
Genotype:  p.[Asp303Asn]+[=]

Interpretation:
One pathogenic mutation has been identified.  The diagnosis of CAPS is confirmed
Genetic counseling is advised to resolve the parental origin of this mutation.

Date of report
Name of the biologist(s)