Reverse-hybridization teststrips for detection of common CYP21A2 mutations in dried blood spots from newborns with elevated 17-OH progesterone

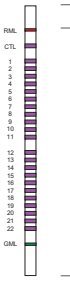
Stefan Németh[1], Gernot Kriegshäuser[1], Sabina Baumgartner-Parzer[2], Franz Waldhauser[3], Stefan Riedl[3], Christian Oberkanins[1]

[1]ViennaLab Diagnostics GmbH, Vienna, Austria, [2]Department of Internal Medicine III, Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria, [3]Department of Pediatrics, Medical University of Vienna, Vienna, Austria.

INTRODUCTION

The average incidence of classical Congenital Adrenal Hyperplasia (CAH) is about 1 in 15,000 births worldwide, and more than 90% are caused by mutations in or deletions of the CYP21A2 gene encoding steroid 21-hydroxylase. In the majority of cases, low/abolished residual enzymatic activity leads to a severe clinical phenotype (salt-wasting CAH) due to aldosterone deficiency. In affected males, clinical signs may not be evident or overlooked at birth, and a life-threatening condition due to salt-wasting crisis during the first weeks of life may occur. For this reason, newborn screening programs based on 17-hydroxyprogesterone (17-OHP) levels have been introduced in various countries to identify affected babies prior to developing a salt-wasting crisis. However, neonatal 17-OHP screening has a considerable false positive recall rate due to several confounding factors (e.g. prematurity, low birth weight, age at sampling, stress, cross-reactivity of immunological methods) causing a substantial economical burden and emotional stress for parents. In Austria, for example, one would expect 6 CAH cases per year, but recall is required in 550 children (0.72%) despite adjustment of 17-OHP cut-off levels to both birth weight and age at sampling. We have therefore developed a reverse-hybridization assay (CAH StripAssay) for rapid simultaneous analysis of common CYP21A2 mutations from dried blood spots in newborns with elevated 17-OHP.

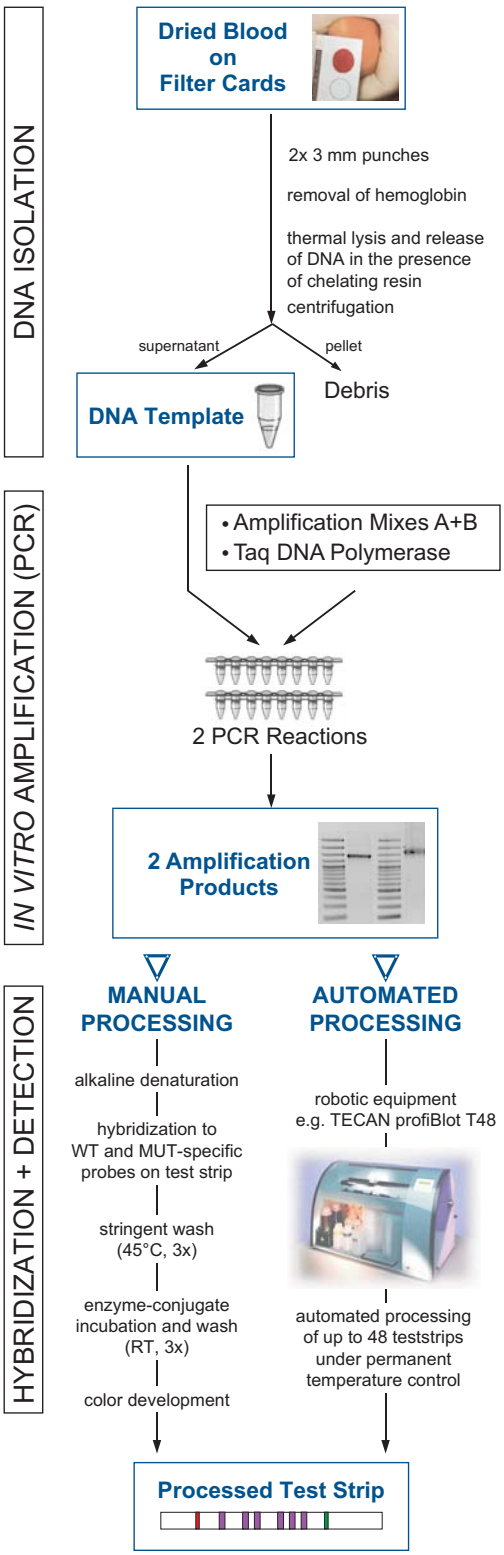
TESTSTRIP DESIGN



LINE	PROBE SPECIFITY	LINE	PROBE SPECIFITY
RML	red marker line (top)	12	wild type P30L
CTL	positive control	13	wild type I2
1	mutant P30L	14	wild type 8 bp del
2	mutant I2	15	wild type I172N
3	mutant 8 bp del	16	wild type Cluster Ex6
4	mutant I172N	17	wild type V281L
5	mutant Cluster Ex6	18	wild type F306+1nt
6	mutant V281L	19	wild type Q318X
7	mutant F306+1nt	20	wild type R356W
8	mutant Q318X	21	wild type P453S
9	mutant R356W	22	wild type R483P
10	mutant P453S	GML	green marker line (bottom)
11	mutant R483P		

Fig.1: Oligonucleotide probe specificities

ASSAY PROCEDURE



METHODS

The CAH StripAssay, applicable for use in dried blood spots, works as follows: The entire CYP21A2 gene is amplified in two overlapping fragments using PCR primers that will not co-amplify the highly homologous pseudogene (CYP21A1P). Biotinylated amplicons are hybridized under exactly defined stringency to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for the following 11 mutations: P30L, I2, 8 bp del (exon 3), I172N, I236N/V237E/M239K ("cluster ex 6"), V281L, F306+1nt, Q318X, R356W, P453S and R483P. Specifically bound PCR products are detected using enzymatic colour reaction. The entire procedure from DNA extraction to the interpretation of results takes less than 8 hours.

RESULTS AND CONLCUSIONS

The new CAH StripAssay was validated in a series of DNA samples of known CYP21A2 genotype. By using the StripAssay in combination with a real-time PCR approach, all 11 covered mutations plus CYP21A2 copy number variations and chimeric genes could be unambiguously identified. Automated instrumentation and the use of a scanner-based software tool (StripAssay Evaluator) for recording and interpreting results may further contribute to making the StripAssay a useful tool in CAH newborn screening programs.

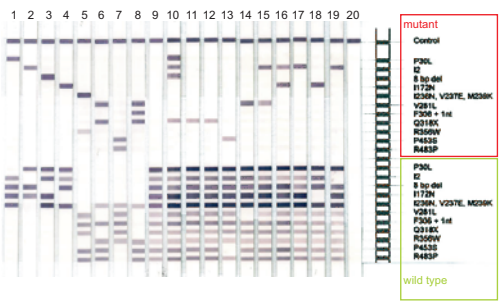


Fig.2: Image of staining patterns obtained with the CAH StripAssay
strips 1-8: recombinant plasmid clones of mutant alleles
strips 9-19: various genomic DNA samples
strip 20: negative PCR control

Dr. Christian Oberkanins, ViennaLab Diagnostics GmbH, Gaudenzdorfer Guertel 43-45, A-1120 Vienna, Austria
phone: +43-1-8120156-40 fax: +43-1-8120156-19 e-mail: oberkanins@viennalab.co.at www.viennalab.com