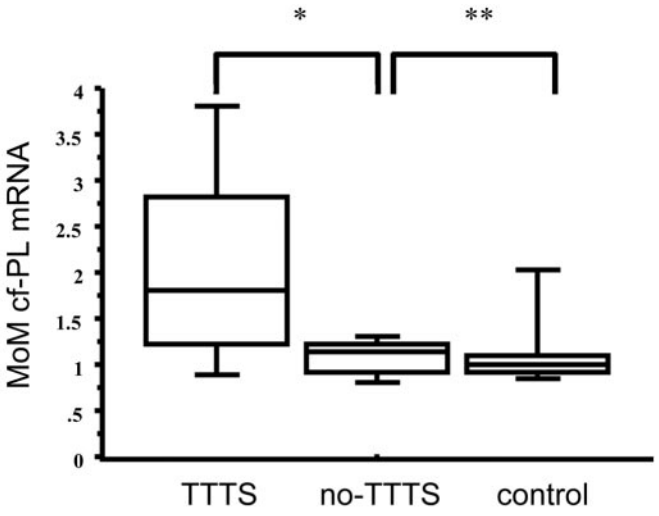

Fig. 1. Box and whiskers plots of cf-PL MoM distribution in the TTTS group, no-TTTS group, and control group.

The median (minimum–maximum) cf-PL mRNA MoM values were 1.80 (0.89–3.81) in the TTTS group, 1.14 (0.77–1.35) in the no-TTTS group, and 1.00 (0.82–2.05) in the control group. * P = 0.035, ** P = 0.41.

group (2.20; range 1.30–2.68) than in the no-TTTS group (1.09; range 0.68–3.25; P = 0.045). Our results suggested the possibility that unapparent pathophysiological changes had already occurred in the women who subsequently developed TTTS, although which specific conditions led to the increased mRNA in the maternal plasma in the TTTS group remain unknown.

In conclusion, a quantitative aberration of both the cf-PL and cf-GAPDH mRNA in maternal circulation may be a novel predictive marker for TTTS, although both statistical differences were small and the sample size was too small to give sufficient strength to the analysis. Therefore, a combination of several cell-free placental mRNA markers could be effective for the prediction of TTTS, similar to the situation for tumor markers. Further study to identify gene transcripts that are expressed only in the placenta and not in blood cells may help to both predict and prevent TTTS and also may further elucidate the pathophysiology of this serious complication.

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Toenails: An Easily Accessible and Long-Term Stable Source of DNA for Genetic Analyses in Large-Scale Epidemiological Studies

To the Editor:

Molecular tools are increasingly applied in epidemiological studies to unravel the relationship between environmental exposures and disease *(1)*. DNA is required for analyses of genetic factors, such as polymorphisms, but currently used specimens, such as lymphocytes and buccal cells, have disadvantages related to collection, transport, storage, and processing of samples. A relatively infrequently used source of DNA that may overcome these problems is nail material. Human toenails have been collected in several epidemiological studies, predominantly for determination of trace elements as biomarkers for the intake of these compounds *(2)*. Until now, none of these epidemiological studies have applied human toenails as a source of DNA.

We investigated whether toenail material collected 20 years ago in the Netherlands Cohort Study on Diet and Cancer (NLCS) *(3)* (n = 120 852) could be used as a source of DNA for analyses of multiple genetic polymorphisms. Approximately 90 000 participants provided toenail clippings (on average, 80 mg per participant) *(2)*. We optimized a protocol for DNA isolation from ~10 mg toenail material, based on the method of Cline et al. *(4)*, and tested the suitability of this DNA in 2 PCR-based

Table 1. DNA yield and multiplex genotyping results.

Material[a]	Toenails n	DNA yield, ng/10 mg toenail		Success rate of multiplex genotyping[b] n (%)	Corresponding buccal swabs available[c] n	Genotype identical[d] n (%)
		Mean (SD)	Range			
Fresh material, <3 months old	11	2102 (669)	1147–3114	11 (100%)		NA[e]
NLCS material, 20 years old						
Irradiated	24	2013 (1167)	1156–7037	23 (96%)	23	22 (96%)
Not irradiated	33	2173 (1623)	950–8929	31 (94%)	12	12 (100%)
Total NLCS	57	2106 (1439)	950–8929	54 (95%)	35	34 (97%)

[a] Material: NLCS material, toenail material collected in the Netherlands Cohort study on Diet and Cancer; Irradiated: toenail material irradiated with neutrons to determine selenium content (Instrumental Neutron Activation Analysis).

[b] Samples in which all 10 polymorphisms could be identified were classified as successful.

[c] Number of toenail samples for which a comparison with buccal swab samples was feasible.

[d] Individuals for whom all 10 polymorphisms in the toenail sample were identical to those in the buccal swab sample.

[e] NA, Not applicable.

assays in a subgroup of the cohort (n = 57) for which buccal DNA was also available. In the 1st assay, 10 single nucleotide polymorphisms were amplified in a multiplex PCR reaction and subsequently genotyped by means of single base extension using primer extension and automated capillary gel electrophoresis as described by Knaapen et al. *(5)*. A 2nd PCR-based test was used to investigate the maximum length of fragments that could be amplified. A portion of the toenail samples had been irradiated with neutrons for analyses of selenium content (not irradiated: n = 33; irradiated: n = 24). To assess possible effects of age of the toenails on the quality of the DNA, freshly harvested toenail material from healthy non-NLCS volunteers was investigated (n = 11) (see the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol53/issue6 for a detailed description of material and methods).

DNA was successfully isolated from all toenail samples. On average, 2 μg DNA could be extracted from 10 mg toenail material (Table 1). There was no significant difference in DNA quantity between the various groups of toenail samples. The large range in DNA yield was mainly due to 2 outliers. The minimum amount isolated was almost 1 μg DNA/10 mg toenail material, which is sufficient for more than 10 multiplex genotyping analyses (80 ng of DNA is sufficient per analysis).

A summary of quantitative results of the multiplex genotyping assays on the DNA samples is provided in Table 1. A genotype profile of 10 polymorphisms was successfully generated for 100%, 96%, and 94% of the DNA samples isolated from fresh, 20-year-old irradiated, and 20-year-old not irradiated toenail material, respectively (amplicon sizes 92–148 bp). The success rate using the buccal swab DNA samples was 100%. Because DNA isolation was successful for only 90% of the buccal swab material in the NLCS samples, the use of nail material as a source of DNA resulted in a higher rate of successful outcomes. For 35 individuals, we compared the outcome of the genotyping assay for both their buccal swab DNA and toenail material DNA. Surprisingly, for 1 person, the 2 sources of DNA resulted in different genetic profiles. This result could not be related to technical errors, but may be from the switching or mislabeling of a sample during the collection process 20 years ago.

The NLCS study received approval by the Medical Ethical Committees of the University Hospital Maastricht and TNO Quality of Life, Zeist, The Netherlands. Study participants gave informed consent.

Results from the 2nd PCR test showed that DNA isolated from 20-year-old (not irradiated) toenail material or from fresh toenail material could be amplified to ≤596 bp. With DNA from 20-year-old irradiated nail material, generation of the 596-bp amplicon was unsuccessful for 60% of the samples, indicating increased fragmentation of the DNA. Irradiation probably causes fragmentation of the DNA, an effect that must be taken into account when the available nail material has previously been used for trace element analyses, which require irradiation.

In conclusion, we showed that 20-year-old nonirradiated and irradiated toenails can be a source of DNA for state of the art high-throughput genetic analyses of polymorphisms. For existing large-scale epidemiological studies, our results demonstrate that toenail material can be used for genetic analyses in cohorts for which no other source of DNA is available. The use of toenails as source of DNA may be of considerable relevance in future molecular epidemiological studies, because toenail clippings can be stored for long periods at low costs while DNA quality remains constant.

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Correction

The article by John Middleton and Jeffrey E. Vaks entitled "Evaluation of Assigned Value Uncertainty for Complex Calibrator Value Assignment Processes: A Prealbumin Example" (Clin Chem 2007;53:735–41), contains several errors. In the first section of *Results*, page 739, the text should be changed from "The IWC value assignment results are shown in Table 1" to "The IWC level 8 results are given in Table 1". Also, in Table 2, for IWC5 to IWC8, the decimal point in the uncertainty SD's should be shifted one digit to the left. Additionally, in Table 3, footnote b, the reference interval should be changed from ". . . based on width of 20 mg/L of the. . . 18–38 mg/L" to ". . . based on width of 200 mg/L of the . . . 180–380 mg/L." Also, in Table 3, the superscript "a" should be removed from the 180 and 380 mg/L table entries. Finally, in the 4th paragraph in *Results* (page 739), the text should be changed from ". . . we made 48 measurements (4 instruments, 12 replicates per sample) . . . to ". . . we made 96 measurements (4 instruments, 24 replicates per sample) . . . ." The authors regret the errors.

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