

Automated DNA isolation for databasing purposes

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Abstract: Nowadays, automation is crucial in databasing laboratories especially for countries which are relative new in the field and must quickly reach the minimum standards of consecrated databasing laboratories from Europe and USA. In our efforts to supply genetic profiles for Romanian National DNA Database, we evaluated the Freedom Evo 150 liquid handling platform with AGOWA sep9600 magnetic separator in different running conditions and workflows, for reaching the most efficient ones. We compared the results with those obtained from manual DNA isolation with Chelex resin and paramagnetic particles. For this we used a total of 6246 biological reference samples (5220 - automated processed and 1026 - manually processed), amplified using AmpFlstr Identifiler Kit and analyzed by capillary electrophoresis using ABI Prism 3100 Genetic Analyzer.

Key words: automation, databasing laboratory, liquid handling platform, DNA magnetic separator.

In the last few years, automation has been successfully implemented in databasing or overcharged forensic DNA laboratories at different sample processing levels: automated gel image or capillary signal analysis systems [1], automated liquid handling platforms, automated PCR setup [2], laboratory information management systems [3].

Automation, in case of sample processing using liquid handling platforms, facilitates the processing of a great number of casework or databasing biological samples per time period and in the same time like any new technological approach, it rises new and specific difficulties of sample management.

The challenge of this approach is to have a robust liquid handling platform as base and to use different technological components in creating highly efficient hybrid robots suitable for forensic DNA analysis. We have tested and optimized such a robot, composed of a Freedom EVO 150 liquid handling platform (**Tecan, Schweiz, AG, Switzerland**) and an AGOWA sep9600 magnetic separator (**AGOWA GmbH, Berlin, Germany**) - (with AGOWA mag DNA Isolation Kit Sputum).

For our evaluation we choose six parameters which could influence the final quantity and quality of the results: sample type, DNA isolation protocol, DNA isolation method, DNA quantity, automated DNA elution/dilution and PCR mastermix DNA concentration.

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MATERIALS AND METHODS

Freedom Evo 150 deck configuration - The Freedom EVO 150 configuration with AGOWA sep9600 includes a 150 cm deck with an 8-channel liquid-handling (LiHa) arm, an AGOWA sep9600 magnetic separator, disposable troughs for reagents of 20 and 100 ml, disposable tips, 96-well plates, racks for 1,5 ml tubes and plate racks (Fig 1). Freedom Evo 150 consumables per run (96 samples): 528 conductive disposable tips – 200 µl, 3 disposable troughs for reagents - 20ml and 2 disposable troughs for reagents – 100 ml (Tecan, Schweiz, AG, Switzerland); 12 thin wall 8 tube strip - 0,5 ml; one 1,5 tube; one deep well plate - 2,2 ml and one 1-96 v-thin wall MT plate (Biozym GesmbH, Austria); 96 tips – 200 µl and 3 tips – 1000 µl.

Reference Biological Samples Preparation – Main part of reference sample processed in our databasing laboratory consists in epithelial cells from saliva collected with Tatort buccal swab kit (Ysselbach, Wien, Austria) and Tatort kit swabs are immersed in 1,5 ml tube with isopropyl alcohol for preservation. For each sample, isopropyl alcohol was eliminated and the swab was dried at room temperature over night. In case of other biological reference sample types (e.g. blood, tissue), the samples were washed once or more if required with sterile water.

Magnetic DNA Isolation Method – We used an adaptation of AGOWA mag DNA Isolation Kit Sputum (10x96) for automated sample processing:

a) **Cell Lysis** - we choose to make this step manually in 1,5 ml tubes by adding 500 µl Lysis Buffer BL and 10 µl Proteinase K for each dried swab followed by 30-120 min./56° C incubation, because of some undesirable incidents encountered when making the cell lysis directly in 2,2 ml Deep Well Platte (e.g. expanded swabs clinging on the conductive tips)

b) **DNA binding on AGOWA mag Particles** - 50 µl lysate, 120 µl Binding Buffer BL and 10 µl AGOWA mag Particles Suspension BL was added in new 0,5 ml tubes; 2 min. room temperature incubation; 1 min. separation with AGOWA sep9600 followed by supernatant removing;

c) **Washing** - 130 µl Wash Buffer BL1 was added on the paramagnetic particles; 1 min. separation with AGOWA sep9600 followed by supernatant removing and a repeated washing step with 70 µl Wash Buffer BL2; 5 min./55°C pellet drying;

d) **Elution** - 30-120 µl of Elution Buffer BL was added on the dried particles; 3 min. separation with AGOWA sep9600; 50 µl supernatant containing purified DNA, was used for dilution or directly for PCR amplification.

DNA extraction results using Freedom EVO 150 with AGOWA sep9600, were compared with those obtained using a classical Chelex method [4].

Quantification - 180 biological samples were quantified (for evaluating the overall quantity of DNA and the paramagnetic particle sedimentation process), using TaqMan® Fast Universal PCR Master Mix and a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

PCR - 2µl and 4.75µl DNA extract for 13,5 µl mastermix total volume, were amplified using the commercial typing kit, AmpF/STR Identifier™ PCR Amplification Kit and a 9700 PE Thermal Cycler (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

Capillary Electrophoresis – ABI 3100 (Genetic Analyzer) and reference sequenced ladders (Applied Biosystems, Foster City, CA, USA).

Software Resources - Freedom EVOware Standard v1.2, Tecan Instrument Software v5.0, Magnetic Separator AGOWA sep9600 Software v2.0, GeneScan Software v3.7.1; Genotyper Software v3.7; 7500 Fast Real Time PCR System Sequence Detection Software v1.3.1.

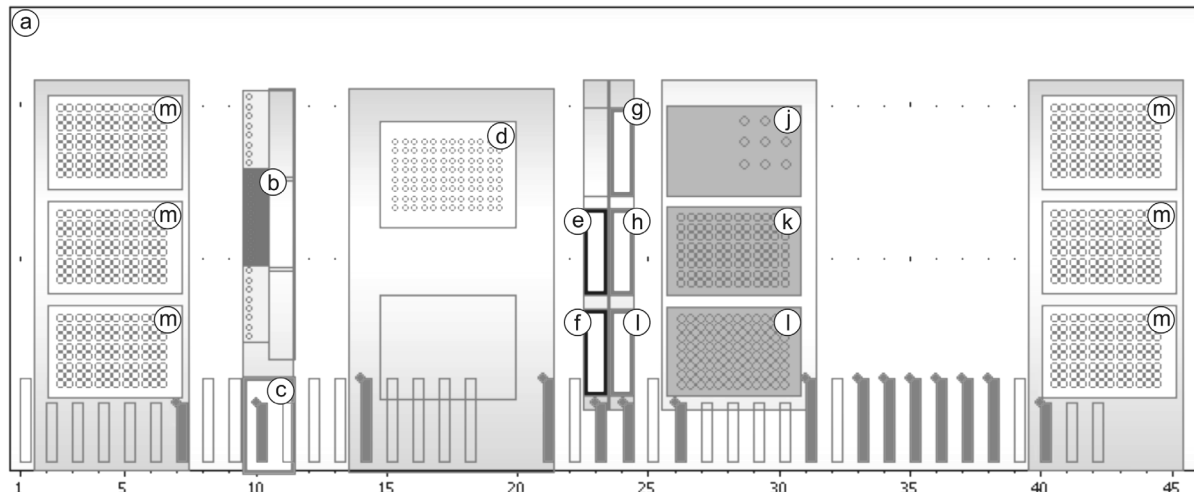


Figure 1 Freedom EVO 150 deck with AGOWA sep9600 - a) standard Freedom Evo 150 worktable, b) washing unit, c) Diti waste, d) AGOWA sep9600, e) Wash Buffer BL1 100ml trough disposable, f) Wash Buffer BL2 – 100 ml trough disposable, g) Binding Buffer BL 20 ml trough disposable, h) Elution Buffer BL 20 ml trough disposable, i) sterile water 20 ml trough disposable, j) Proteinase K tube holder trough, k) DNA extract - 96 v-thin wall MT plate, l) Lysate - 2,2 ml deep well plate, m) 1x96 conductive disposable tips (200 µl)

EVALUATION METHODOLOGY AND PARAMETERS

We evaluated a total of 6246 biological reference sample: 5220 processed with Freedom Evo and 1026 manually processed; 486 - processed samples using Chelex method and 5788 – processed using magnetic method; 5184 - buccal swab samples and 36 samples other than saliva (blood, tissue and hair) processed with Freedom Evo; 450 samples processed using Chelex method and 2610 using magnetic method (two concentrations for PCR amplification: 2 and 4,75 µl DNA extract); 3878 - 1/5 dilution (chelex and magnetic) manually processed; 360 manually and 5428 automated processed using magnetic method; 1350 - 1/3 automated eluted and 720 - 1/5 automated eluted (magnetic method).

We also analyzed 3100 ABI Prism electropherograms with peak heights threshold of 150 rfu. Non-rendering off-alleles and alleles under 150 rfu were considered 0. In case of imbalanced peak heights for heterozygous loci with one allele under 150 rfu, we removed the entire locus.

RESULTS AND DISCUSSIONS

1. Sample Type

For evaluation we separate the biological reference samples in two main types: buccal swabs and other sample types. Difference between these two categories (14,56% for complete genetic profiles – Fig. 2), could result from the fact that Lysis Buffer in case of or other unspecific reference sample type (hair, blood, tissue) is added according to the sample quality and quantity. As a general rule we always add 500 µl for buccal swabs and between 50 µl and 300 µl for other sample types.

2. DNA Isolation Protocol

Automated biological samples processing is more efficient than manual processing, first by quality (13,29% more efficient for complete genetic profiles – Fig. 3), second by processing time (96 samples in 2-3h), third by improving work conditions (e.g. reducing contact with toxic reagents) and fourth by minimizing direct and cross-sample contamination risk.

3. DNA Isolation Method

The Chelex method is one of the most popular methods used in forensic genetics because it is easy and fast. Despite that, it seems to be easier to implement a magnetic method on a liquid handling platform than a Chelex one, because centrifugation steps are replaced with a magnetic field modulation. Also, one of the major benefits of the magnetic method is the purity of magnetic DNA extracts, higher than that obtained with a Chelex method, which needs an additional step for purification.

Another benefit, is the elimination of the incubation step (8 min./100⁰ C), where tubes have the risk to explode because of alcohol traces. rising the risk of cross-sample contamination. In our evaluation, the magnetic method is 6,09% more efficient than the Chelex method. If it is made with a liquid handling platform such as Freedom Evo 150 the magnetic method is even more efficient (14,53%) (Fig. 3).

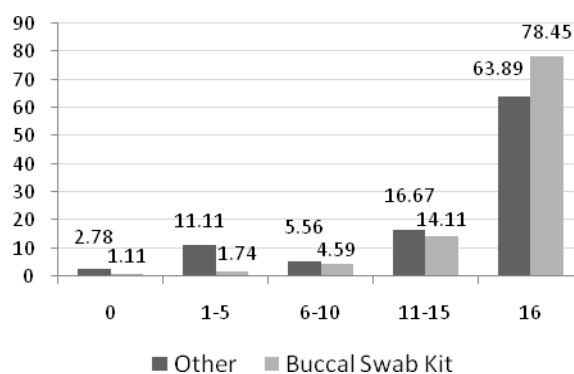


Figure 2 DNA isolation success rate (%) by sample type, using Chelex and magnetic methods and manual and automated protocols (where 0 to 16 are the number of amplified STR loci).

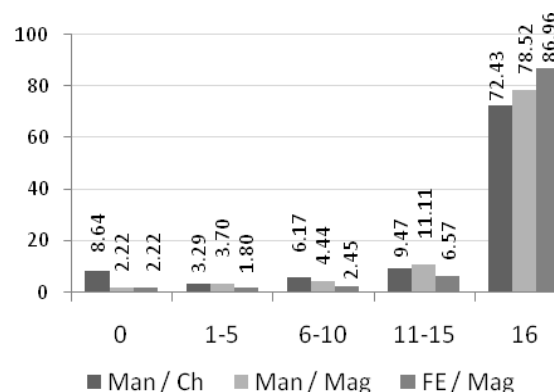


Figure 3 DNA isolation success rate (%) by method and protocol (where 0 to 16 are the number of amplified STR loci, Man. – manual, Mag – Magnetic, Ch – Chelex, FE – Freedom Evo 150).

4. DNA Quantity

Using Freedom EVO with AGOWA 9600 Sep and AGOWA mag DNA Isolation Kit Sputum, the average DNA quantity obtained from buccal swab was 550 ng/ μ (Fig 4).

We noticed a magnetic particle sedimentation process occurring between container mixing step (single tube with ~1 ml paramagnetic particles) and the discharging of 10 μ l paramagnetic particles in each sample tube distributed on AGOWA sep9600. This sedimentation process influences the final DNA quantity by creating variations between samples and also between 8-tube strips (our robot works with 8 conductive tips at once).

From our measurements, 10 μ l of AGOWA mag Particles Suspension have an average of 0,63 mg dried paramagnetic particles. Considering the manufacturer value of 2 μ g DNA/mg particles, it results in theory that 1260 ng is the maximal amount of DNA that can be retained on the surface of the paramagnetic particles.

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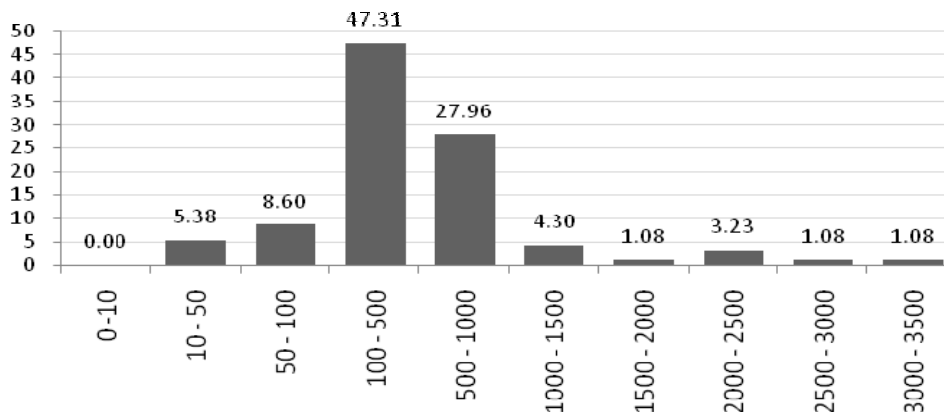


Figure 4 Human DNA quantity (%) obtained from buccal swabs, using Freedom EVO with AGOWA 9600 Sep and AGOWA mag DNA Isolation Kit Sputum. (where 0-3500 are ng/μL values).

From our measurements, $10 \mu\text{l}$ of AGOWA mag Particles Suspension have an average of $0,63 \text{ mg}$ dried paramagnetic particles. Considering the manufacturer value of $2 \mu\text{g}$ DNA/mg particles, it results in theory that 1260 ng is the maximal amount of DNA that can be retained on the surface of the paramagnetic particles.

To determine the sedimentation process impact on the DNA quantity, we calculated the average of the DNA quantity for each 8 strip line and visualized the results in two ways:

a) with all values (Fig. 5) and

b) with values under 1260 ng , considering values bigger than 1260 ng as being Real Time PCR reading errors (Fig 6).

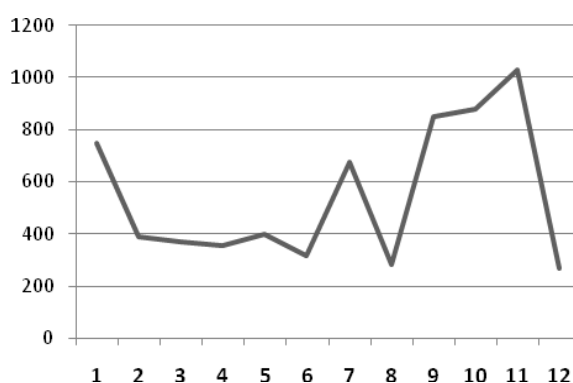


Figure 5 Human DNA quantity (ng/μL) variation caused by paramagnetic particles sedimentation, for all sampled (where 1-12 are samples grouped by plate strip number)

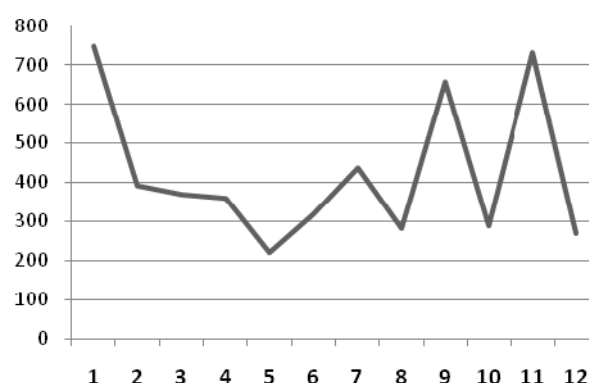


Figure 6 Human DNA quantity (ng/μL) variation caused by paramagnetic particles sedimentation, for samples with values lower or equal than the max. capacity value (where 1-12 are samples grouped by plate strip number)

It seems that the sedimentation process occurs upon half of the paramagnetic particles quantity or half of the time of paramagnetic particles distribution on AGOWA

sep9600, because afterwards the conductive tips reach the sediment where the paramagnetic particles concentration is higher than the rest.

5. Automated DNA Elution/Dilution

One major problem of automation was the high concentration of the DNA extracts. If we take in consideration the manufacturer DNA concentration values for PCR amplification using the AmpF/str Identifier Kit (10 µL DNA extract with ~1 ng/µL DNA concentration [5]), we should dilute the DNA extracts hundreds of times (Fig. 5).

Because we work with 96 gaps plates we can't do that without losing samples (not all extracts have the same DNA quantity).

This is why, based on accumulated experience, we used for elution 90 µl and 120 µl of Elution Buffer. Because the Elution Buffer quantity provided in AGOWA mag DNA Isolation Kit was insufficient, we performed the elution with 70 µl Elution Buffer, followed by dilution, using sterile water (Fig. 7 and 8). Elution step followed by dilution with sterile water didn't influence the final results.

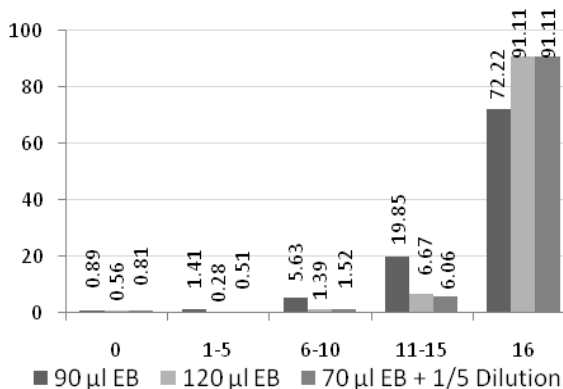


Figure 7 DNA isolation success rate (%) for Elutions/Dilutions made with Freedom Evo 150 (where 0 to 16 are the number of amplified STR loci and EB – Elution Buffer)

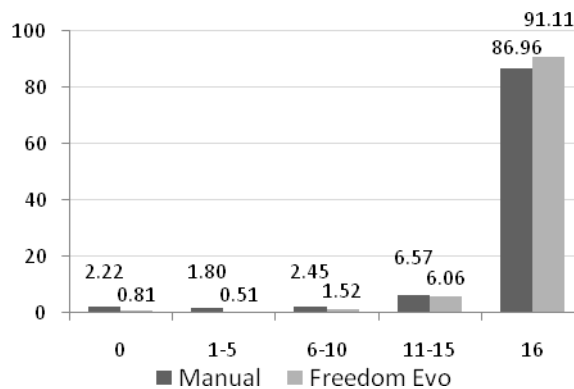


Figure 8 DNA isolation success rate (%) by protocol, using 70µl Elution Buffer and 1/5 Dilution (where 0 to 16 are the number of amplified STR loci)

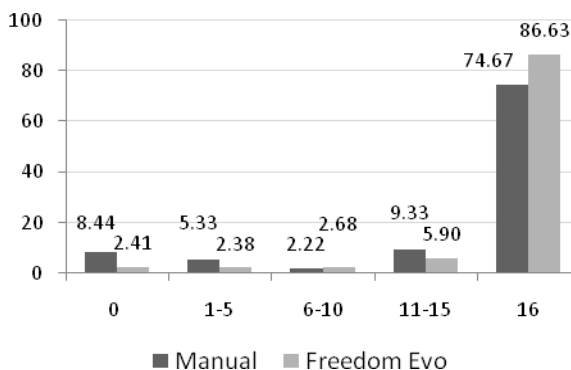


Figure 9 DNA isolation success (%) rate for 2 µl DNA volume in the PCR mastermix (where 0 to 16 are the number of amplified STR loci).

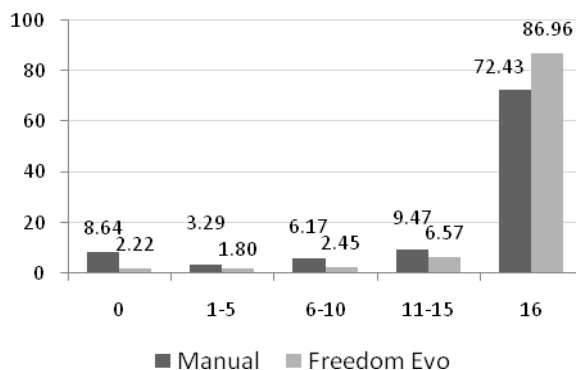


Figure 10 DNA isolation success rate (%) for 4.75 µl DNA volume in the PCR mastermix (where 0 to 16 are the number of amplified STR loci)

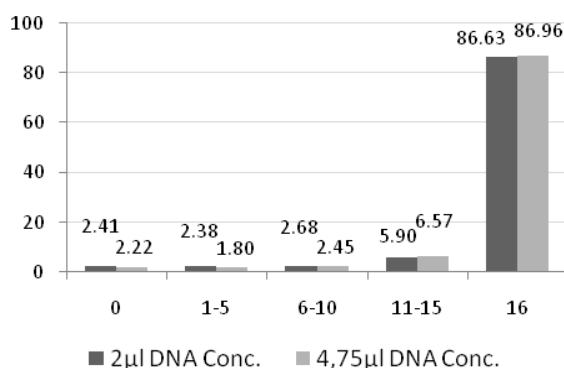


Figure 11 DNA isolation success rate (%) for 2 and 4.75 µl DNA concentration in PCR mastermix using Freedom Evo 150 (where 0 to 16 are the number of amplified STR loci)

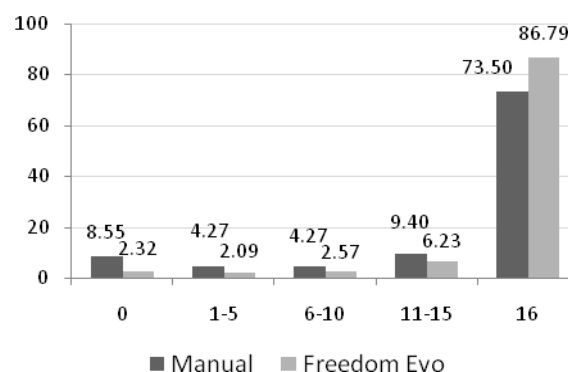


Figure 12 DNA isolation success rate (%) by PCR mastermix DNA concentration average (where 0 to 16 are the number of amplified STR loci)

6. PCR Mastermix DNA Concentration

For reproducibility reasons, we performed two PCR amplifications for each sample. Overtime, we observed that there are no major differences between results.

We also noticed that the lowest was the DNA volume (e.g. 1-2 µl per mastermix total volume) the higher was the degree of pipetting errors.

This is the reason why we choose to amplify only one volume for the usual samples (4,75 µl DNA extract) and two or more different volumes for the difficult samples (which generate incomplete DNA profiles) (Fig. 9, 10, 11 and 12).

CONCLUSIONS

From all evaluated parameters, 91,11% was the uppermost success rate achieved using: automated magnetic DNA isolation method (AGOWA mag DNA Isolation Kit Sputum) with Freedom Evo 150, buccal swab samples, elution with 70 µl Elution Buffer, 1/5 dilution and 4,75 µl DNA concentration in PCR mastermix, per sample.

We consider the rest of 8,99% as being the results of the following factors (in the most probable order): low quality sampling, manual pipetting errors and PCR or capillary electrophoresis failures.

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DISCLAIMER

Authors report no conflict of interest relevant to their findings, products or producers mentioned in this article.

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