

# Automated DNA extraction from large volume samples using the InviGenius®

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## ABSTRACT

The analysis of genetic information continues to gain importance in all aspects of life ranging from individual ancestry research to pharmacogenomic studies in drug development. Often such analysis needs large amounts of DNA for downstream analysis e.g. next generation sequencing. In this study samples such as blood or saliva are purified using a new magnetic particle processor which features a reliable pipetting tool for variable sample volumes from 200 to 4000 µl. The unique instrument automates diagnostic extraction protocols for nucleic acids with full in-process control even for volumes higher than 1 ml. The instrument is able to accommodate protocols with up to 17 ml in total volume using standard plastics. Up to 150 µg DNA were isolated per preparation in the automated process depending on the nature of sample and on the donor. Data are shown for DNA extraction from up to 4 ml of human EDTA stabilized blood and from 1.6 ml of stabilized saliva samples. Saliva was previously collected by the newly designed SalivaGene system which stabilizes DNA in saliva samples for one year at ambient temperatures. Yield and purity of the extracted DNA is suitable for various downstream applications based on enzymatic amplification and detection of DNA.

## METHODS

### 1) Samples

Blood samples were stabilized with EDTA and stored at 4° C or at - 20° C. Saliva samples were stabilized using the SalivaGene® system (2 ml of saliva) and stored at room temperature.

### 2) Extraction Kits

a) InviMag® SalivaGene DNA Kit, b) InviMag® Blood DNA Giga Kit, c) InviMag® Blood DNA Mini Kit

All extraction procedures were performed using standard scripts on the InviGenius® instrument.

### 3) Real-time PCR

- Template amount 2 µl, reaction volume 12.5 µl
- Amplification profile: 95 °C, 5 min - 40 cycles; 95 °C, 10 s / 58 °C, 20 s  
Product: GAP-DH, size of the product: 192 bp

## RESULTS

### 1) Reproducibility and sample comparison

24 samples from 6 different bloods and 24 samples from 2 saliva pools were extracted. All samples were measured by a NanoDrop photometer for absorptions at 230, 260, 280 and 320 nm. For 12 blood samples the InviMag® Blood DNA Mini Kit/ IG and for the other 12 samples the InviMag® Blood DNA Giga Kit/ IG was used. Stabilized saliva samples were extracted using the InviMag® SalivaGene DNA Kit/ IG. Each data point represents the average of 4 independent extractions.

Tab. 1: NanoDrop results for blood and saliva samples.

|            | Sample  | 260/230  | 260/280 | Conc. [ng/µl] | Yield [µg] | Elution volume [µl] | yield / ml of sample [µg] | sample volume [µl] |
|------------|---------|----------|---------|---------------|------------|---------------------|---------------------------|--------------------|
| Blood Mini | Blood 1 | 1,1      | 1,8     | 45,15         | 9,0        | 200                 | 45,2                      | 200                |
|            |         | +/- 32 % | +/- 5 % | +/- 9 %       | +/- 9 %    |                     | +/- 9 %                   | 200                |
|            | Blood 2 | 1,0      | 1,8     | 41,80         | 8,4        | 200                 | 41,8                      | 200                |
|            |         | +/- 26 % | +/- 4 % | +/- 26 %      | +/- 26 %   |                     | +/- 26 %                  | 200                |
|            | Blood 3 | 1,0      | 1,9     | 25,10         | 5,0        | 200                 | 25,1                      | 200                |
|            |         | +/- 29 % | +/- 5 % | +/- 12 %      | +/- 12 %   |                     | +/- 12 %                  | 200                |
| Blood Giga | Blood 4 | 1,3      | 1,8     | 80,13         | 64,1       | 800                 | 16,0                      | 4000               |
|            |         | +/- 11 % | +/- 2 % | +/- 15 %      | +/- 15 %   |                     | +/- 15 %                  | 4000               |
|            | Blood 5 | 1,3      | 1,7     | 173,80        | 69,5       | 400                 | 17,4                      | 4000               |
|            |         | +/- 11 % | +/- 2 % | +/- 8 %       | +/- 8 %    |                     | +/- 8 %                   | 4000               |
|            | Blood 6 | 1,4      | 1,8     | 167,98        | 134,4      | 800                 | 33,6                      | 4000               |
|            |         | +/- 7 %  | +/- 1 % | +/- 22 %      | +/- 22 %   |                     | +/- 22 %                  | 4000               |
| Saliva     | Pool 1  | 1,0      | 1,6     | 84,3          | 42,2       | 500                 | 26,4                      | 1600               |
|            |         | +/- 2 %  | +/- 1 % | +/- 7 %       | +/- 7 %    |                     | +/- 7 %                   | 1600               |
|            | Pool 2  | 0,8      | 1,6     | 148,3         | 74,8       | 500                 | 46,7                      | 1600               |
|            |         | +/- 1 %  | +/- 1 % | +/- 13 %      | +/- 13 %   |                     | +/- 13 %                  | 1600               |

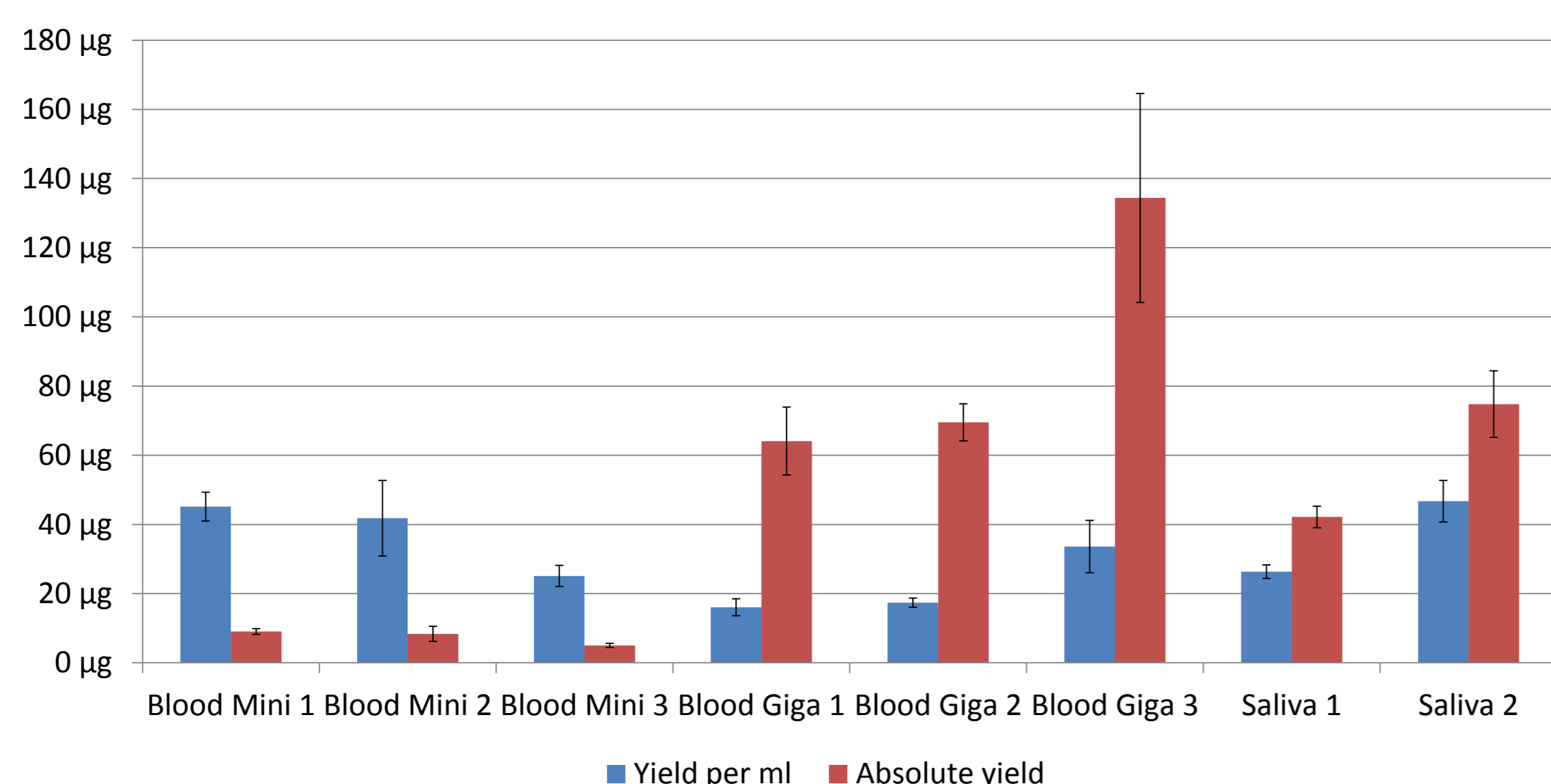


Fig. 1: The blood and saliva samples delivered highly reproducible results. The small sample volume protocol (200 µl) for blood samples gave a slightly higher yield (µg DNA/ ml) in comparison to the large volume protocol (4 ml). A total yield of 50 µg – 140 µg DNA was obtained using the InviMag® Blood DNA Giga Kit/ IG. The saliva protocol from 1.6 ml of starting material resulted in approx. 40 µg – 100 µg DNA. All protocols provided high DNA yields in high quality. Therefore the DNA amount is suitable for a variety of downstream applications which need large amounts of template DNA, e.g. next generation sequencing.

### 2) Cross contamination test - InviMag® Blood DNA Giga Kit

After experiment 1 genomic DNA was isolated from 6 different blood samples on the same instrument in 4 subsequent runs. Between the samples always negative samples without blood were positioned. All samples were extracted and measured by a NanoDrop photometer for absorptions at 230, 260, 280 and 320 nm. The samples were analyzed by an agarose gel. An aliquot of the samples was used for a real-time PCR of the GAP-DH pseudogene (Data not shown).

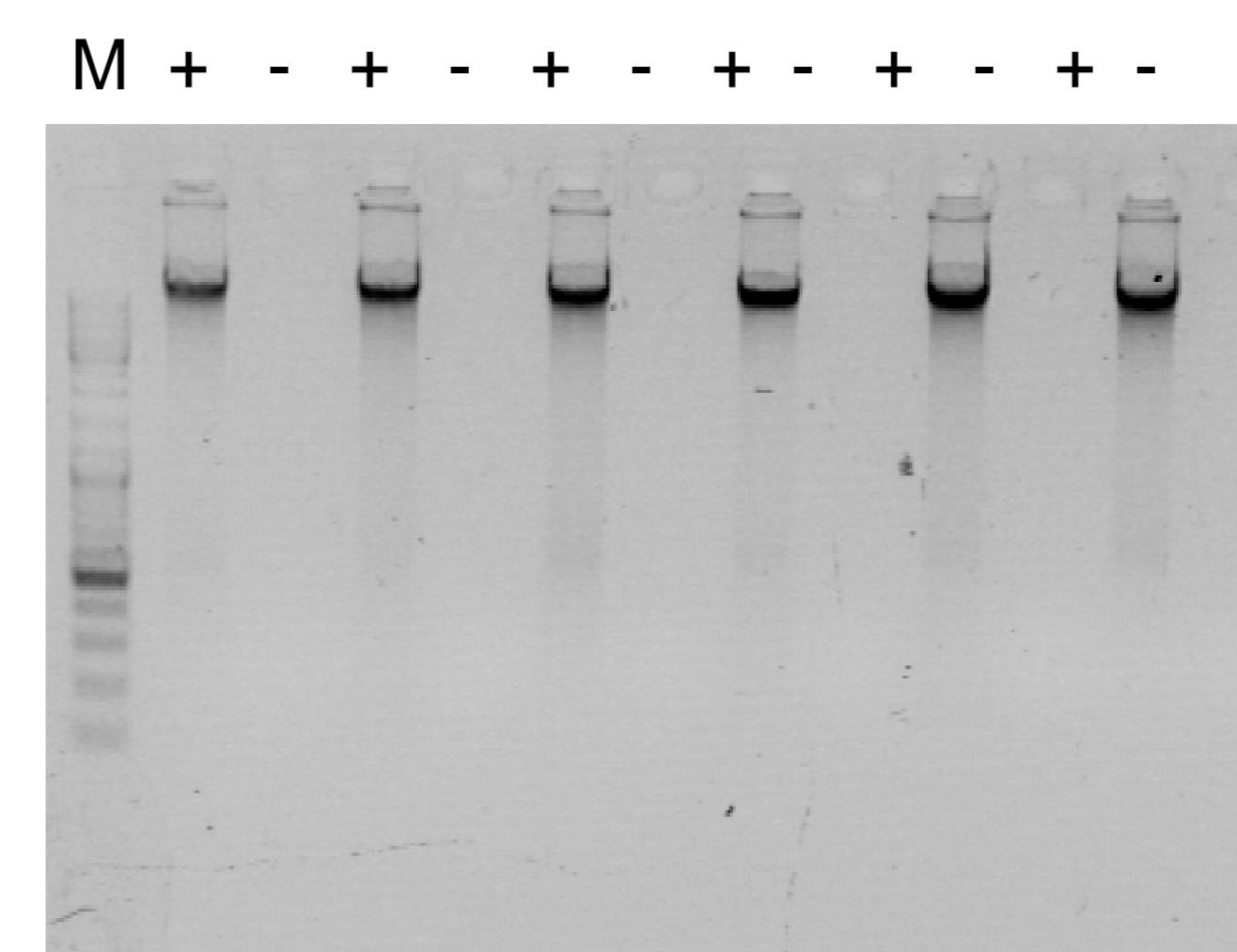


Fig. 2: A representative agarose gel picture is shown. All positive samples showed absorptions at 260 nm, clear distinct bands on the gel, as far as Ct values between 22 and 28. The negative samples showed no absorption at 260 nm, no band on the gel and also no amplification signal.

### 3) Variability with different samples - InviMag® SalivaGene DNA Kit

Genomic DNA was isolated from 12 different stabilized saliva samples on the InviGenius® in two runs, each with 6 samples in duplicates. All samples were measured using a NanoDrop photometer for absorptions at 230, 260, 280 and 320 nm. The samples were also analyzed by agarose gel electrophoresis (data not shown). An aliquot of each eluate was used for a real-time PCR reaction for the GAP-DH pseudogene.

Tab. 2: Mean values of NanoDrop and real-time PCR results for saliva samples

| Sample    | OD260 | OD280 | purity | yield [µg] | Ct    |
|-----------|-------|-------|--------|------------|-------|
| Saliva 1  | 4,755 | 3,195 | 1,6    | 104,5      | 20,49 |
| Saliva 2  | 1,65  | 0,98  | 1,735  | 39,3       | 21,1  |
| Saliva 3  | 1,335 | 0,835 | 1,69   | 30,45      | 22,34 |
| Saliva 4  | 1,005 | 0,59  | 1,76   | 24,1       | 21,96 |
| Saliva 5  | 1,705 | 1,095 | 1,635  | 39,15      | 19,21 |
| Saliva 6  | 0,67  | 0,38  | 1,825  | 16,15      | 22,35 |
| Saliva 7  | 4,26  | 2,9   | 1,565  | 93,85      | 20,66 |
| Saliva 8  | 1,14  | 0,62  | 1,89   | 27,95      | 22,3  |
| Saliva 9  | 0,505 | 0,305 | 1,825  | 11,3       | 22,85 |
| Saliva 10 | 1,395 | 0,89  | 1,645  | 32         | 21,23 |
| Saliva 11 | 0,68  | 0,355 | 1,925  | 17,1       | 18,98 |
| Saliva 12 | 0,865 | 0,49  | 1,795  | 21,1       | 22,7  |
| PTC       | -     | -     | -      | -          | 23,05 |

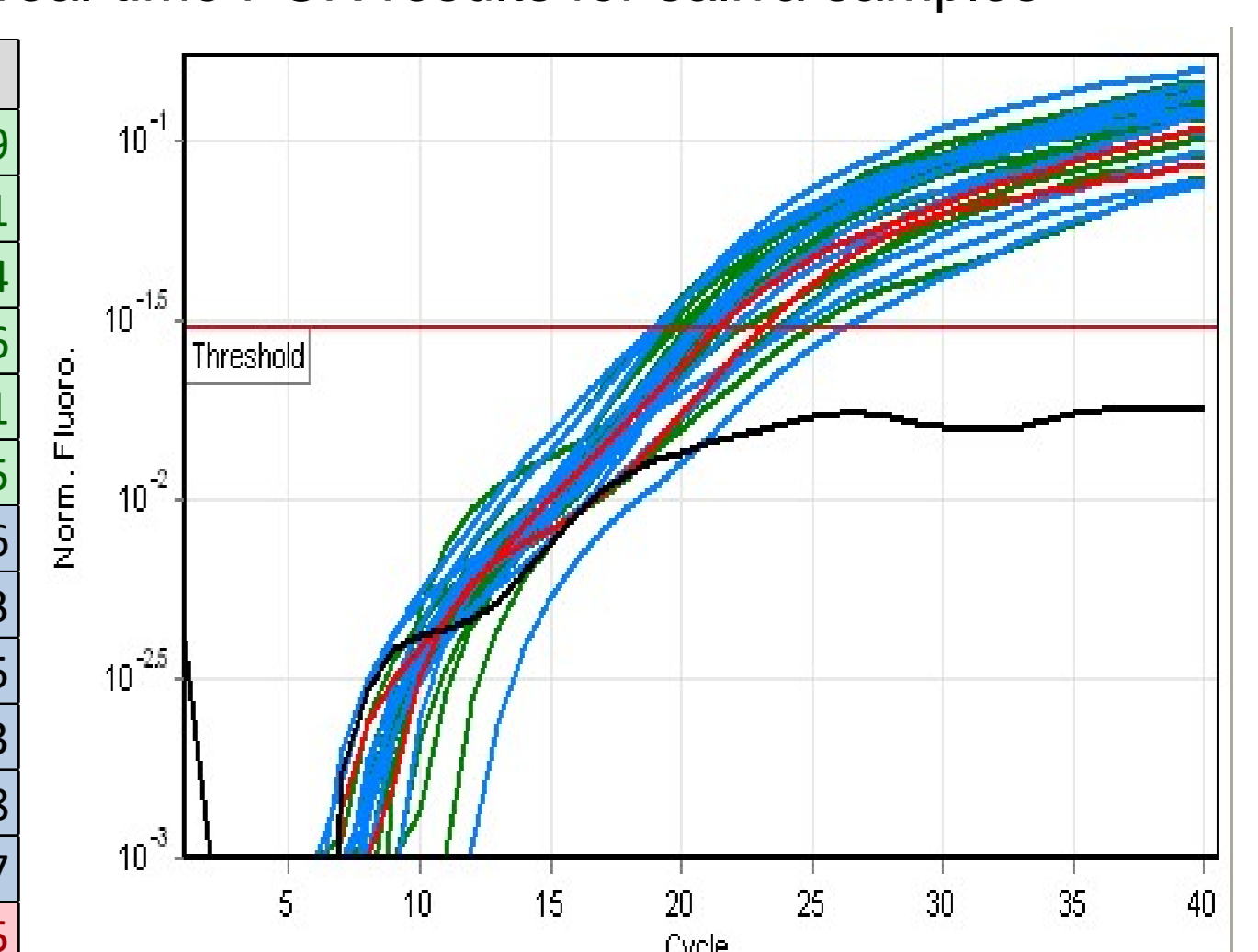


Fig. 3: All samples showed yields between 9 µg and 115 µg DNA and clear distinct bands on the agarose gel (not shown). All samples showed amplification signals in the real-time PCR (Ct values between 19 and 26). In the table mean values are shown.

## SUMMARY

Samples such as blood or saliva were purified using the InviGenius® magnetic particle processor for variable sample volumes from 200 to 4000 µl. DNA was successfully extracted from different samples and volumes. Standard sample volume (200 µl) resulted in yields in the range of 5 - 10 µg with excellent purity. The higher volume protocols delivered DNA amounts ranging from 16 - 50 µg of DNA per ml of sample. Saliva samples differed in DNA content from 10 µg/ml to 200 µg/ml. The variability in blood samples is lower. All high volume protocols gave excellent DNA quality based on spectrophotometric analysis and real-time PCR. Tests proved that the InviGenius® instrument shows no detectable cross-contamination.