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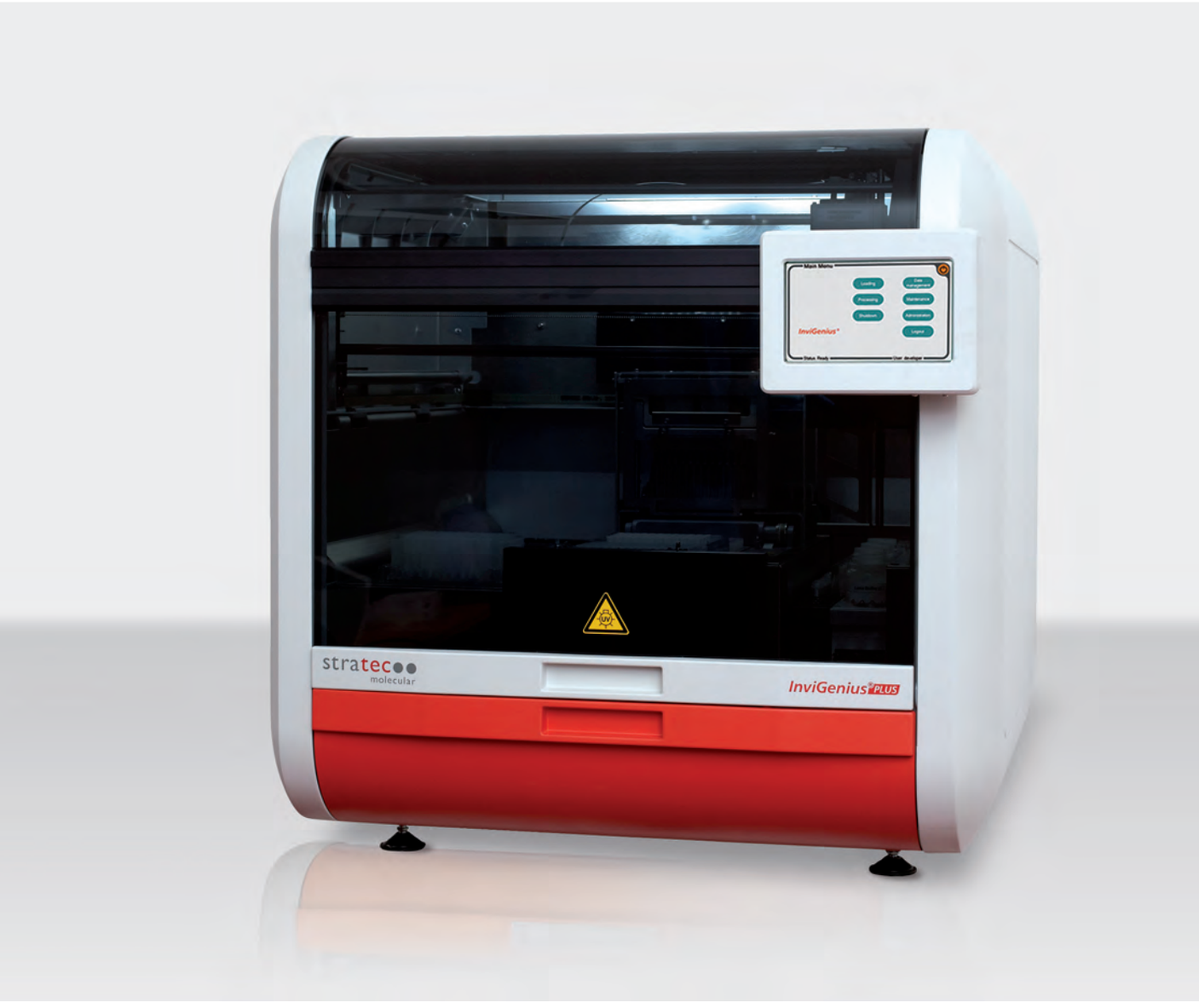
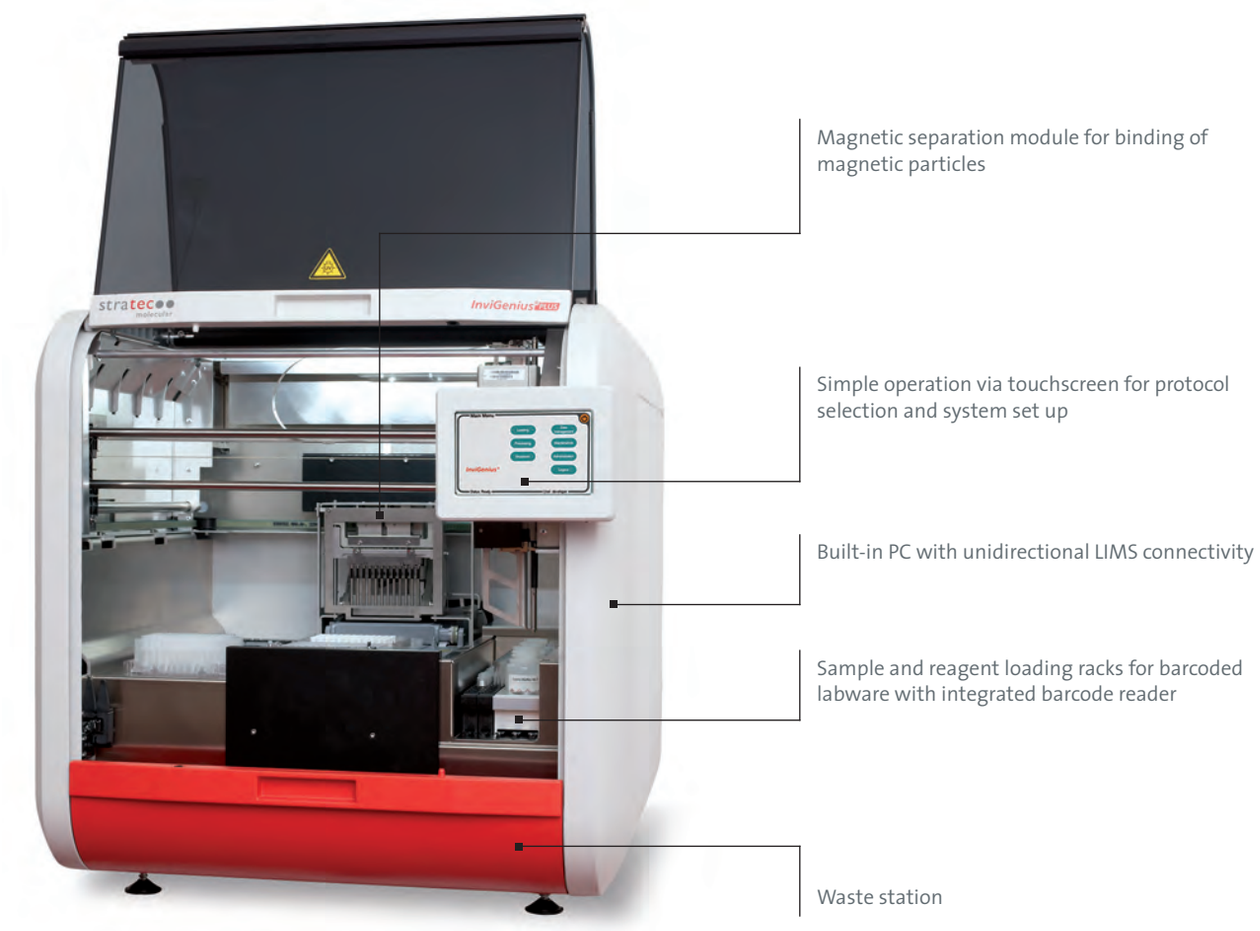


The InviGenius® PLUS

Walk-away DNA/RNA sample preparation

CONTENT

Superior automated sample preparation



The InviGenius® PLUS is a walk-away system for nucleic acid purification for up to 12 samples from up to 4 ml of sample volume. Innovative functionality and optimized protocols for demanding samples and applications result in reliable performance and superior DNA and RNA quality for molecular diagnostics*.
The combination of well-established magnetic bead based InviMag® technology and state-of-the-art process automation allows for standardization and streamlining of laboratory workflows.

**) In compliance with the Directive 98/79/EC on in vitro diagnostic medical devices (IVD-Directive). Products which are CE-marked according to the IVD-Directive can be used for diagnostic applications in countries where this directive is recognized. The device is not approved by the US FDA.*

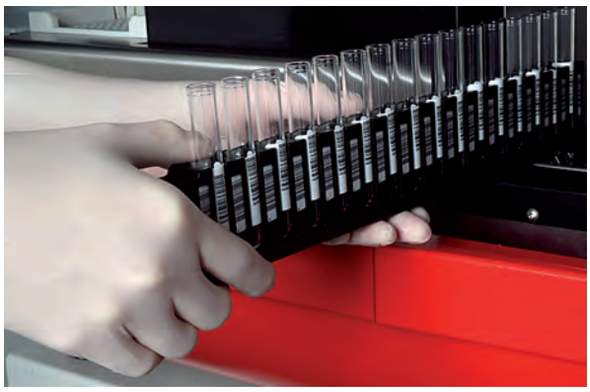
InviGenius® PLUS deck layout

The new PLUS features

1. Deep-well working plate for sample volumes of up to 4 ml
2. Heat lysis and heat elution for high recovery rates of nucleic acids from demanding samples such as liquid biopsies or virus samples
3. Barcoded labware for complete sample traceability (e.g. 2 ml barcoded elution tubes)
4. Choice of elution tubes or plates for flexible sample management
5. Droplet catcher minimizes the risk of cross contamination

Ultimate safety of set-up and operation

The InviGenius® PLUS delivers exceptional process safety in a completely monitored operation environment. Elaborate software control and intuitive user guidance enable full process control and documentation and prevent sample tracking errors.



1. Loading racks for primary tubes

- Integrated barcode reader for sample identification during loading
  - Prevention of human errors
  - Tracking of samples
  - Connectivity with LIMS allowing workflow continuity and archiving of the data

Examples of compatible primary tubes:

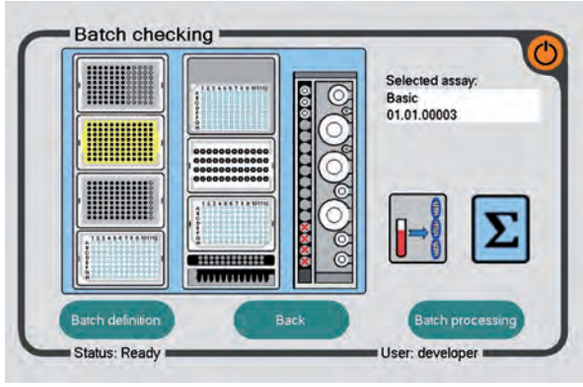
- Sarstedt Monovette®
- BD Vacutainer®
- TERUMO Venosafe®
- Greiner Bio-One Vacuette®

2. Loading rack with flexible reagent positioning

- Automated reagent detection – independent of position
  - Prevention of human errors
  - Verification of correct reagents
  - Verification of valid expiration dates

3. Plug-in for separate hand-held barcode scanner

- Choice of elution tubes or plates
- Complete traceability for all plastic ware



4. Software-supported setup of reagents, waste and disposables

- Inventory checks before and during run
- Defined and protected protocols prevent user-errors

5. Air displacement pipettor

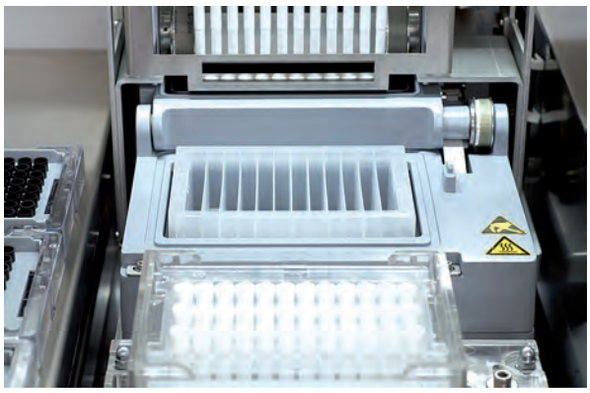
- Barometric and conductive liquid level detection
- Reduced maintenance and waste - no system liquid is required
- Prevention of cross contamination through use of filter tips and intelligent routing

Additional pipetting options:

- Aliquot pipetting of eluates for generation of replicates, e.g. for PCR
- Pre-mix of samples to prevent clogging and for sample resuspension

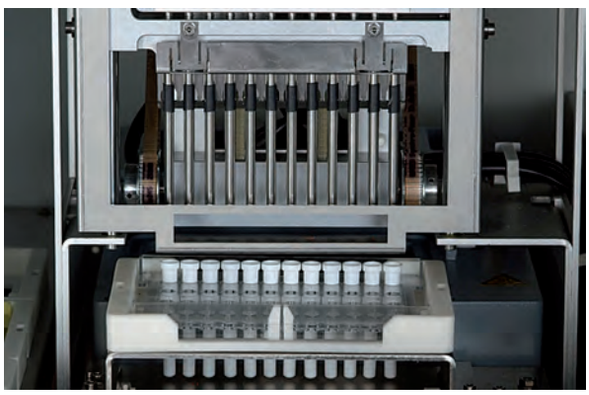
Fully automated, walk-away DNA/ RNA purification

The InviGenius® PLUS is a true walk-away system for DNA/RNA extraction and purification from clinical samples – providing a reliable "Sample in – Eluate out" technology!



1. Heat incubator

- Automated heat lysis (up to 95 °C) on board
- A heated lid prevents aerosol formation and condensation
- Suitable for deep-well working plate for sample volumes of up to 4 ml



2. Magnetic separation module

- Twelve magnetic rods transfer magnetic beads and mix samples
- Disposable sheath racks for several runs
- Bottom magnets prevent carry-over of beads into the eluate
- Droplet catcher prevents cross-contamination

3. Waste management and decontamination

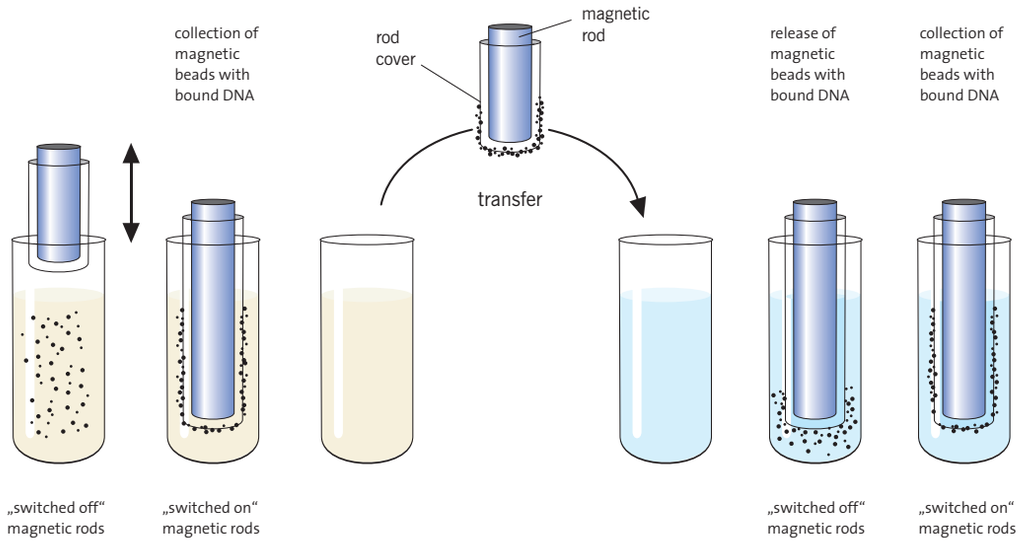
- Used tips and sheaths will be disposed into the waste container; Pipettor drips excessive liquid into it
- Waste container is rotating slowly for better utilization and can be removed and disposed
- UV light enables decontamination of the worktable

Advanced troubleshooting:

- Acoustic signal, e.g. in case of closed bottles, clotted sample
- Flagging of problematic samples, e.g. clots
- Automatic hard disk space management

Nucleic acid extraction principle

The InviGenius® PLUS controls an array of magnetic rods that can collect or release magnetic particles. After sample lysis the nucleic acids are bound to the magnetic particles and transferred through the extraction, purification, and elution processes. This circumvents pipetting errors. The eluted pure nucleic acids are ready-to-use for subsequent downstream applications.



The InviMag® technology for the InviGenius® PLUS system increases laboratory efficiency by minimizing the need for individual kits for different applications for a broad range of starting materials. Sample volumes up to 4 ml can be processed, providing superior sensitivity for genomic applications and pathogen detection.

Starting materials

Whole blood, serum, plasma, urine, stool*, sputum*, BAL, swab, saliva, transport media

**) Sample pretreatment is necessary*

Target nucleic acids

Genomic DNA, cell-free circulating DNA, RNA & DNA from viruses, bacterial DNA

Kits and applications

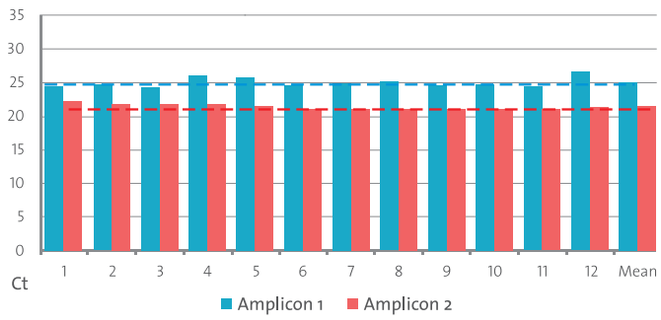
NUCLEIC ACID	STARTING MATERIAL	PRODUCT NAME
Genomic, bacterial, viral DNA & viral RNA	200 µl whole human blood (EDTA, citrate), plasma, serum, cell-free body fluids, rinse liquid from swabs, supernatant from stool suspensions, sputum, BAL, urine	InviMag® Universal Kit/ IG
Viral and bacterial DNA & viral RNA	500 - 1000 µl plasma, serum, cell-free body fluids, rinse liquid from swabs	InviMag® Virus Midi Kit/ IG (available from Q1 2017)
Genomic DNA	200 µl whole human blood (EDTA, citrate) 2 ml whole human blood (EDTA, citrate)	InviMag® Blood DNA Mini Kit/ IG InviMag® Blood DNA Maxi Kit/ IG
	1.6 ml SalivaGene® stabilized saliva samples	InviMag® SalivaGene DNA Kit/ IG
	4 ml plasma, serum, urine	InviMag® Free Circulating DNA Kit/ IG
Bisulfite converted DNA	500 pg to 5 µg of genomic DNA , cfDNA, FFPE DNA (20 - 50 µl eluted DNA)	InviMag® Bisulfite Conversion Kit/ IG

The InviMag® technology incorporates many years of experience in developing magnetic bead based kits for automated systems. The InviMag® kits rely upon STRATEC Molecular proprietary formulations and ensure superior results for the extraction and purification of DNA and RNA.

1. Automated extraction of circulating cell-free DNA

The **InviMag® Free Circulating DNA Kit/ IG** enables efficient, fully automated purification of cell-free circulating DNA (cfDNA) fragments from 4 ml of plasma or serum samples on the InviGenius® PLUS.

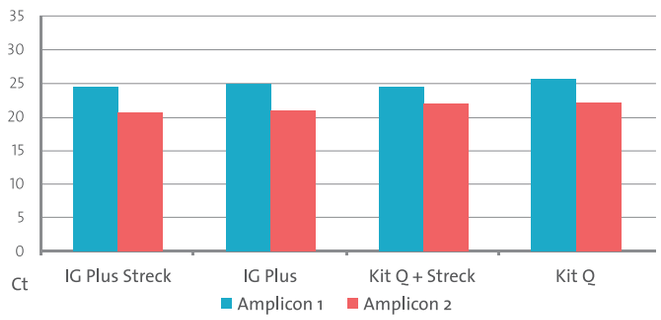
Fig. 1: Excellent and reproducible intra-run recovery of circulating cfDNA



*Circulating cell-free DNA was isolated from 12 aliquotes of 4 ml Seracon plasma samples in parallel using the **InviMag® Free Circulating DNA Kit/ IG** on the **InviGenius® PLUS** and eluted in 100 µl. DNA yield was quantified by real-time PCR of two amplicons within the 18S rRNA sequence.*

- FAM (70 bp) CT Std. Dev: 0.72
- Cy5 (177 bp) CT Std. Dev: 0.33

Fig. 2: Comparison of extraction efficiency using automated and manual kits

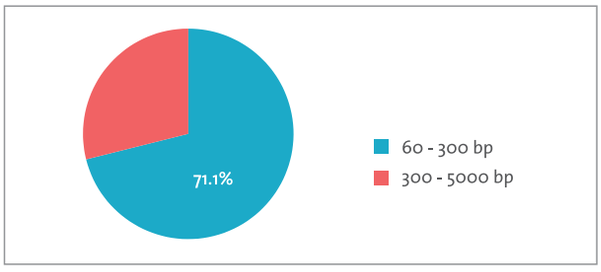


*cfDNA was isolated from 4 ml plasma (same blood collected in Streck tubes and EDTA tubes) using the fully automated procedure of the **InviMag® Free Circulating DNA Kit/ IG** on the **InviGenius® PLUS** in comparison to a manual kit. DNA was quantified by real-time PCR of two amplicons within the 18S rRNA sequence (70 bp & 177 bp). Samples collected with Streck tubes yield comparable results to conventional EDTA tubes and the extraction efficiency is at least comparable to competition.*

- FAM (70 bp) CT Std. Dev: 0.72
- Cy5 (177 bp) CT Std. Dev: 0.33

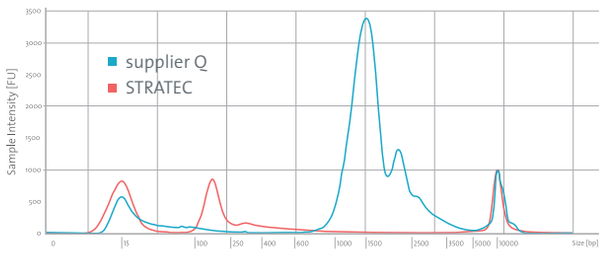
Fig. 3 a+b: cfDNA fragment lengths distribution using automated & manual extraction methods

Automated - InviMag® FreeCirculating DNA Kit/ IG



*3a. cfDNA was isolated from 40 different plasma samples of patients diagnosed with cancer of different origin and stages using the **InviMag® Free Circulating DNA Kit/ IG** on the **InviGenius® PLUS.***
*The automated kit on the **InviGenius® PLUS** yields a proportion of over 70% small cfDNA fragments from 60 – 300 bp which are of higher interest for subsequent biomarker analysis in oncology.*

Bioanalyzer fragment lengths distribution



*3b. Example of cfDNA fragment lengths analysis using the Bioanalyzer TapeStation HSD 5000. Comparison of cfDNA extracted from identical sample using the **InviMag® Free Circulating DNA Kit/ IG** on **the InviGenius® PLUS** and the manual kit from supplier Q.*

2. Automated workflow from clinical sample to methylation analysis

The **InviMag® Bisulfite Conversion Kit/ IG** features a fully automated and reliable bisulfite treatment and conversion of cfDNA and genomic DNA from whole blood, serum, plasma, cell cultures, tissue samples and FFPE slides for methylation analysis. The process is fully automated on the InviGenius® PLUS and can be combined with previous extraction workflows on the instrument.

Fig. 1: Automated cfDNA extraction

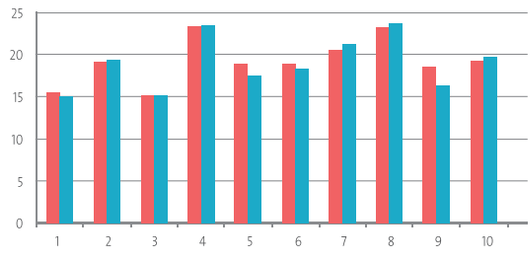


Fig. 1 Comparison of CT values for DNA quantification of an automated & manual cfDNA extraction method.

*cfDNA was isolated from 2 ml of ascites and rinsed cytology samples from ovarian carcinoma patients using the **InviMag® Free Circulating DNA Kit / IG** on the **InviGenius® PLUS** platform and a manual commercial system. After extraction the cfDNA was quantified using the InviQuant GeneCount 40.*

- InviMag® cfDNA Kit/ IG
- Manual cfDNA Kit (supplier Q)

Fig. 2: Automated bisulfite conversion

*Afterwards 50 µl of all eluates were used for the fully automated bisulfite conversion on the **InviGenius® PLUS** in combination with the **InviMag® Bisulfite Conversion Kit/ IG**.*

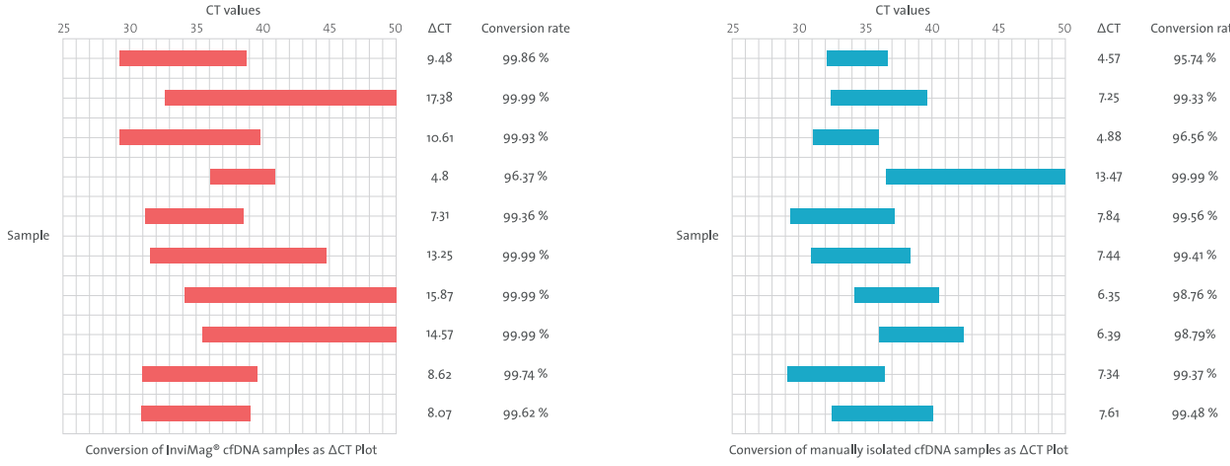


*Fig. 2 shows the conversion efficiency by demonstrating the CT values of the converted DNA versus the unconverted DNA of the automated bisulfite conversion protocol. cfDNA extracted with the automated protocol and the manual kit from Supplier Q respectively was subjected to automated bisulfite conversion on the **InviGenius® PLUS**. The bisulfite conversion is efficient for DNA from different sources (ΔCT > 6 represents a conversion rate of > 99.99 %).*

Fig. 3: Methylation Biomarker analysis

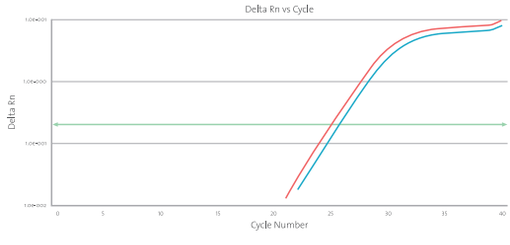


*Fig. 3 Bisulfite converted DNA from the automated protocol was used for detection of an ovarian cancer methylation biomarker by qPCR. Fig. 3 shows a representative result generated with the automated bisulfite conversion protocol comparing cfDNA extracted with a manual (supplier Q) and the automated extraction protocol on the **InviGenius® PLUS**.*

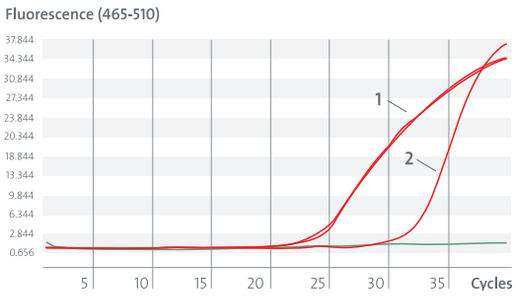
Data kindly provided by Mrs. Hums, oncgnostics GmbH in co-operation with Gynecological Molecular Biology, Department of Gynecology, Jena University Hospital, Germany.

- InviMag® cfDNA Kit/ IG (CT 25.05)
- Manual cfDNA Kit (CT 25.71)

3. One isolation kit for various starting materials and nucleic acid types

The **InviMag® Universal Kit/ IG** enables fully automated purification of genomic, bacterial, viral DNA and viral RNA from a variety of clinical samples on the InviGenius® PLUS with sample volumes of up to 200 µl. Different primary tubes can be placed directly into the sample loading rack.

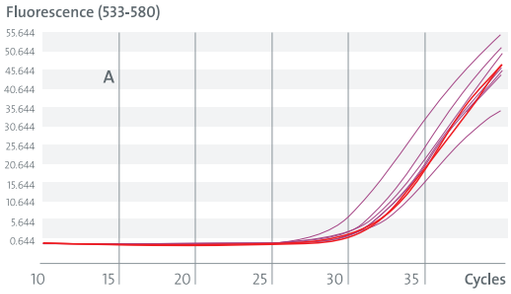
Fig. 1: Viral RNA from sputum

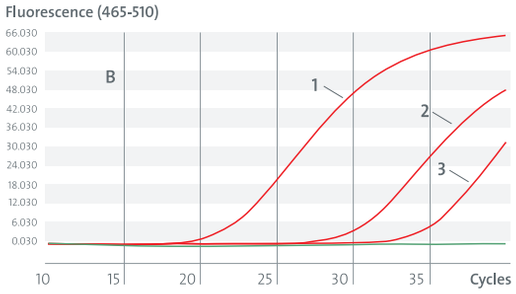


*RNA from human metapneumovirus was isolated from sputum samples using the **InviMag® Universal Kit/ IG** and the spin column based Invisorb® Spin Virus RNA Mini Kit from STRATEC Molecular. 10 µl of the eluted RNA were amplified using the „dia Human metapneumovirus„ assay from Mikrogen Diagnostik (Neuried, Germany). Both extraction methods showed comparable CT values.*

1 - positive patient samples
2 - positive control

Fig. 2: Bacterial DNA isolation (Gram-positive bacteria)

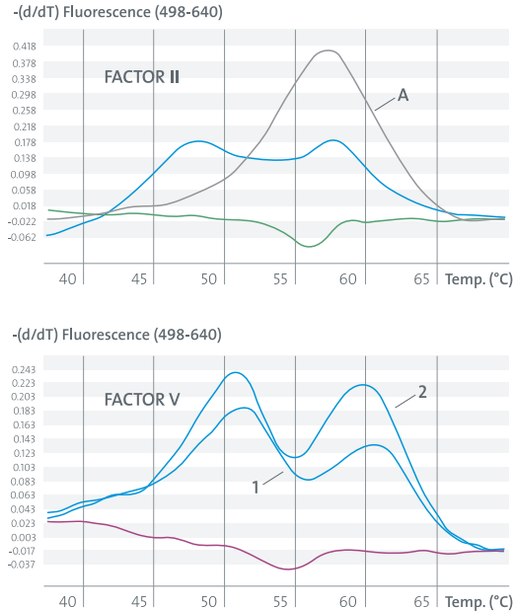




*Bacterial DNA was isolated from twelve potentially infectious patient samples (sputum samples, bacteria culture from swab) using the **InviMag® Universal Kit/ IG**. 10 µl of the eluted DNA were amplified using the „MutaPLATE M. tuberculosis„ real-time PCR assay from Immundiagnostik AG (Bensheim, Germany).*

A: internal controls of all 12 samples – were amplified, without any inhibition
B: red - TBC positive patient samples [swab (1); sputum (2) internal control (3)] green - TBC negative patient samples (valid negative results)

Fig. 3: Genomic DNA isolation from blood samples



*Genomic DNA was isolated from 200 µl of human blood using the **InviMag® Universal Kit/ IG**. 10 µl of isolated DNA were amplified using the „RealStar Faktor II PCR Kit 3.0„ und „RealStar Faktor V PCR Kit 3.0„ from Altona Diagnostics (Hamburg, Germany).*

Factor II: Patient sample - wild-type
Factor V: Patient sample - heterozygous for factor V

Factor II:
green - negative control
blue - positive control
grey - patient sample, wild-type (A)

Factor V:
purple - negative control
blue - positive control (1)
blue - patient sample, heterozygous (2)

Tab. 1: Isolation of nucleic acids from twelve different pathogen containing samples in the same run

*DNA/RNA from different viruses and bacteria species were isolated using the **InviMag® Universal Kit/ IG** in the same run in parallel. For comparison an equivalent STRATEC Molecular spin kit was used with aliquots of the same sample. DNA/RNA eluates were analyzed via real-time PCR using the assays listed below. The results show comparable CT values.*

STARTING MATERIAL	PATHOGEN	SPIN KIT	CT: UNIVERSAL	CT:SPIN	ASSAY
stool*	*Norovirus*	Invisorb® Virus RNA Mini Kit	24.92	27.06	RIDA GENE Norovirus [1]
swab from urethra**	*Neisseria gonorrhoeae*	RTP® Bacteria DNA Mini Kit	21.55	23.12	Neisseria gonorrheae [2]
swab**	*MRSA*	RTP® Bacteria DNA Mini Kit	22.88	23.56	GeneOhm MRSA Kits [3]
stool*	*EHEC*	PSP® Spin Stool DNA Kit	26.12	27.00	RIDA GENE EHEC/EPEC rt PCR [1]
urine	*Chlamydia trachomatis*	RTP® Bacteria DNA Mini Kit	32.71	32.37	Cobas Taqman CT V2.0 [4]
stool*	*Clostridium difficile*	RTP® Bacteria DNA Mini Kit	32.76	30.34	RIDA GENE CD TOX A/B V [1]
sputum****	*Mycobacterium tuberculosis*	RTP® Mycobacteria Kit	27.17	30.46	MTB compl [2]
sputum	*Metapneumo Virus*	Invisorb® Virus RNA Mini Kit	23.79	24.00	Metapneumo Virus [2]
lyophilized cell lysate***	*Influenza A/B (inkl. H1N1 & H5N1)*	Invisorb® Virus RNA Mini Kit	24.73	24.03	Influenza S&T RT PCR Kit2.0 [5]
sputum	*Mycoplasma pneumophila*	RTP® Bacteria DNA Mini Kit	34.32	29.58	Mycoplasma & Pneumophila [2]
swab**	*Adeno-Virus*	Invisorb® Virus DNA Mini Kit	32.00	32.00	Adeno-Virus [2]

**) supernatant from 50 mg stool, resuspended in 600 µl RNAse-free water, centrifuged at 2000 rpm for 2 min*
***) rinsed in 600 µl RNAse-free water*
****) resuspended in 1 ml bidest. water*
*****) sample is mixed with 20 vol % NAC Buffer and incubated for 10 min at 95°C*

1: R-Biopharm
2: Mikrogen
3: BD
4: Roche Diagnostics
5: Altona Diagnostics

All data for the InviMag® Universal Kit/ IG kindly provided by M. Haesner, Medizinisches Labor Prof. Schenk/ Dr. Ansorge, Magdeburg, Germany.

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