A Universal Protocol for the Extraction of Nucleic Acids from Clinical Specimens Adapted to Different Laboratory Automation Platforms

Birgit Höding, Hans Joos, Christian Jurinke, Beate Kraffert, Oliver Krätke, Andrea Ockhardt, Norbert Wendt

STRATEC Molecular GmbH, Berlin, Germany

ABSTRACT

Robust and reliable purification of nucleic acids from diverse and complex clinical samples remains a challenging task for diagnostic applications. Many diagnostic laboratories are faced with the task to extract different nucleic acids from a variety of clinical samples during a typical working shift. To collect batches of similar specimens or to sort by nucleic acid type is not always feasible or desirable. Consequently, different protocols or kits are used for the various applications. A simplification of this practice towards the use of only one unified protocol would reduce the average cost, labor and sources of error. We have developed a reagent set with universal protocols for the parallel extraction of nucleic acids (DNA and RNA) from clinical samples (e.g. EDTA-blood, plasma, urine, sputum). The system is based on either magnetic bead or 96-well filtration plate technologies. The protocols have been adapted to various laboratory automation platforms including the STARlet (Hamilton) the KingFisherÊ (Thermo) and the InviGenius® (Stratec Molecular) and are also amenable to manual Spin-Filter use. The data presented in this poster demonstrate the equivalence of the universal protocol in terms of sensitivity of extracted nucleic acids from clinical samples compared to specific manual methods for the extraction of genomic DNA, viral RNA, and DNA, as well as bacterial DNA. It is furthermore demonstrated that the extracted nucleic acid can be used in different downstream applications for detection and analysis.

METHODS

1) Samples

Cell free body fluids, (serum, plasma, CSF, urine), whole blood (stabilized with EDTA or Citrate, no Heparin) rinse liquid from swabs or transport media, supernatant from stool suspension, sputum, bronchoalveolar lavage (BAL), sperms or semen, amniotic fluid, supernatant from organ abrasion, bacterial or viral cultures. 200 µl of each sample were used per experiment.

2) Extraction Kits

a) InviMag® Universal Kit/ STARlet, b) Invisorb® Universal HTS 96 Kit/ STARlet
c) InviMag® Universal Kit/ IG, d) InviMag® Universal Kit/ KF Flex96,
e) Invisorb® Spin Universal Kit
All extraction procedures were performed according to the kit instructions.

3) Real-Time PCR Detection

All reactions were performed according to the instructions of the manufacturer

RESULTS

1) Comparison of sensitivities

The data presented in Tab. 1 demonstrate the equivalence of the universal protocol in terms of sensitivity of extracted nucleic acids from clinical samples compared to specific manual or automated methods for the extraction of genomic DNA, bacterial DNA, viral RNA, and DNA.

Starting Material	Pathogen - using STARlet	Nucleic acid specific Kits from competitors	InviMag® Universal Kit/ STARlet	Invisorb® Universal Kit/ STARlet
Supernatant from stool susp.	Adenovirus[1]	14.92	14.49	15.41
Swabs from cloaca	Avian corona Virus[2]	16.98	17.08	16.15
Bronchial secrete	*Bordetella pertussis*[1]	24.77	24.98	24.51
Supernatant from stool susp.	*Campylobacter spp.*[1]	23.27	23.27	22.7
Tracheal secrete / BAL	*Mycoplasma pneumonia*[3]	27.61	27.07	26.98
	Pathogen - using InviGenius		**InviMag® Universal Kit/ IG**	
Swab	*MRSA*[4]	23.56	22.88	
Sputum	*Mycobacterium tuberculosis*[5]	30.46	27.17	
Swab	*Neisseria gonorrhoeae*[4]	23.12	21.55	
	Pathogen - using KingFisher Flex 96		**InviMag® Universal Kit/ KF Flex 96**	
Rinsed liquid from swab	Influenza A[6]	26.68	24.91	
Supernatant from stool susp.	Norovirus[1]	26.46	26.52	
Supernatant from stool susp.	*Chlostridium difficile*[1]	29.54	29.18	
	Pathogen - using Spin columns		**Invisorb® Spin Universal Kit**	
Amniotic fluid	Avian Influenza A[2]	32.96	24.51	
Bacterial culture	*Riemerella anatipestifer*[2]	24.03	23.98	

Tab. 1: Samples from different starting materials were divided in identical aliquots in order to directly compare the Universal Kits versus three manual extraction kits from competitors. The resulting nucleic acids are compatible with different commercially available detection kits.

1) R-Biopharm AG, 2) Anicon Labor GmbH, 3) Diagenode s.a., 4) Becton Dickinson
5) Nanogen Advanced Diagnostics S.p.A., 6) altona Diagnostics GmbH

2) Performance comparison for Universal Kit and specialized manual kit

The Universal Kit series delivers robust performance and comparable results to specialized established extraction kits.

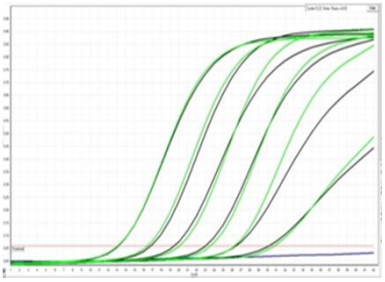

Fig. 1: Avian Influenza A detection
- 200 µl allantoic fluid (infected with Influenza A Subtype H9N2)
- Dilution series: 10 - 10^{-5}
- Invisorb® Universal HTS 96 Kit/ STARlet (black),
- Spin Virus RNA Kit from a competitor (green)
- Detection of Influenza A with Kylt™ Avian Influenza A real-time PCR Detection Kit and Chromo 4, Biorad

3) Sensitive recovery of internal extraction controls

The Universal Kit series allows using internal extraction controls with highly reproducible recovery.

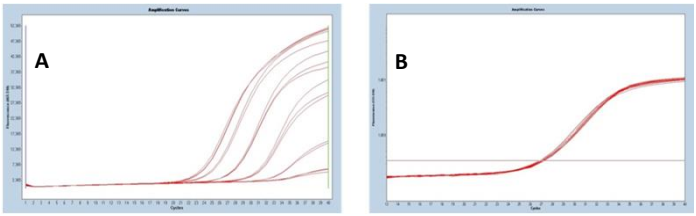

Fig. 2: Norovirus extraction and internal extraction control
Graph A displays Real-Time PCR results from a dilution series of a norovirus sample pool isolated with three different kits (see Tab. 2). Graph B shows Real-Time PCR results from the extraction controls of the respective samples.

Dilution series (FAM)	Manual Spin Kit X	InviMag® Universal Kit/ STARlet	Invisorb® Universal Kit/ STARlet
1:10	27,13	26,44	26,54
1:100	29,86	29,09	28,93
1:1000	31,52	30,52	31,52
1:10000	32,64	32,46	32,36

Tab. 2: Norovirus detection
- 200 µl supernatant from stool suspension
- Dilution series: 10 - 10^{-4}
- Invisorb® & InviMag® Universal Kits/ STARlet
- Spin Virus RNA Kit from a competitor
- Detection: RIDA®GENE Norovirus Assay, R-Biopharm (LightCycler ®, Roche)

SUMMARY

Genomic DNA, viral RNA, viral DNA, and bacterial DNA were simultaneously extracted from diverse and complex clinical samples on different robotic platforms (STARlet (Hamilton) the KingFisherÊ (Thermo) and the InviGenius® (Stratec Molecular) using a uniform extraction method, called the Universal Kit. The protocol is amenable to use in a magnetic bead, filter plate or spin column format. Real-Time PCR assays delivered comparable results in terms of sensitivity for samples and internal controls extracted with specialized manual kits and automated Universal Kits.
The use of a Universal Kit chemistry saves cost and sample processing efforts while delivering robust results.