

Automation of the KYLT[®] RNA / DNA Purification HTP Kit for rapid veterinary diagnostics

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Introduction

The detection and surveillance of veterinary pathogens by molecular analysis is an invaluable tool for risk assessment and the reduction of financial losses. Animal diseases not only cause the loss of productivity but can also pose a threat to food safety. Therefore, constant evaluation of the entire food production chain, from farm to fork, is indispensable for public health.

Conventional culture-based methods are not well suited for the detection of certain bacterial or viral pathogens. In comparison, Real-Time (RT) PCR is advantageous with regard to both sensitivity and specificity and can be performed with fast turnaround times.

As part of the SAN Group, KYLT develops and produces a wide spectrum of (RT-)qPCR-based assays for simplified diagnostics of relevant veterinary and food pathogens. These assays are additionally offered as diagnostic service by ANICON. To cope with the high processing demands sometimes exceeding 600 samples per day, an extremely capable automation solution was developed featuring the integration of the KYLT Purifier Element device.

Here, we present a fully automated solution for

high-throughput sample-to-result workflows using the KYLT RNA / DNA Purification HTP Kit and compatible KYLT (RT-)qPCR assay setup on the Hamilton Microlab STARlet platform.

Method Description

The KYLT RNA / DNA Purification HTP Kit was developed to simplify the first crucial step in any molecular diagnostic application, which is the purification of target nucleic acids from common veterinary diagnostic samples. It facilitates the purification of all relevant nucleic acids (viral or pro- and eucaryotic RNA and DNA) simultaneously from a broad range of sample matrices such as ANICARDS, swabs, tissue, feces, serum, plasma and other bodily fluids derived from different animal species and their environment.

In this Application Note, we have compared results obtained from the previous routine automation solution featuring the Hamilton Heater Shaker Module and Magnetic Plate with the newly developed solution using integrated KYLT Purifier Element technology on the Microlab STARlet.



Figure 1: The Hamilton STARlet Platform with Housing Extension (exploded view).

- Rapid and precise testing of pathogens in livestock and from various sample matrices
- Improved speed and quality through KYLT Purifier Element integration
- Simplified workflow saving time and expense while increasing productivity

System Description

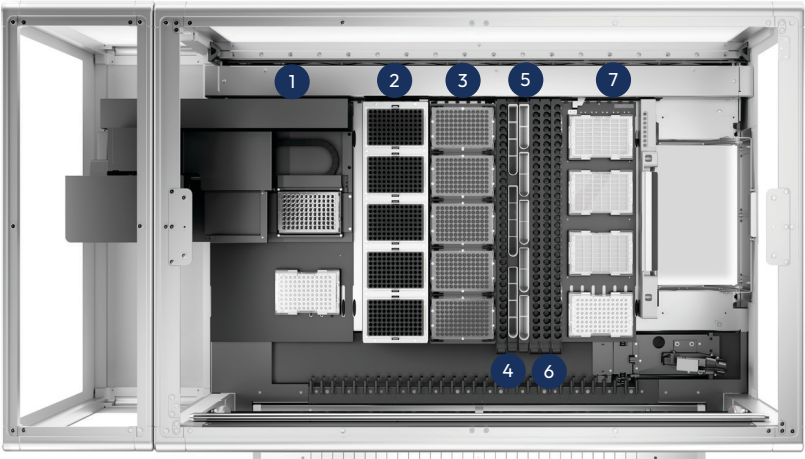


Figure 2: Deck Configuration of the STARlet System (with Housing Extension) for Automating the KYLT RNA / DNA Purification HTP Kit.

- 1 Integration space for KYLT Purifier Element with additional Deep-Well Plate Position used for KYLT Purifier Spin Tips
- 2 Tip Carrier with 3x 1 mL and 2x 300 µL Tip Racks
- 3 Plate Carrier with 4x KYLT Purifier Plates and 1x STARLAB Elution Plate
- 4 Reagent Tube Carrier
- 5 Reagent Trough Carriers
- 6 Sample Tube Carriers
- 7 Carrier with 48-well positions and KYLT Purifier sample plate

Application Software

The connection to and control of the KYLT Purifier Element is realized with the HSL Serial Library used in a separate dedicated sub-method library.

The sub-method library also includes an elaborate error handling method that can recover any potential error at runtime.

Visual Workflow

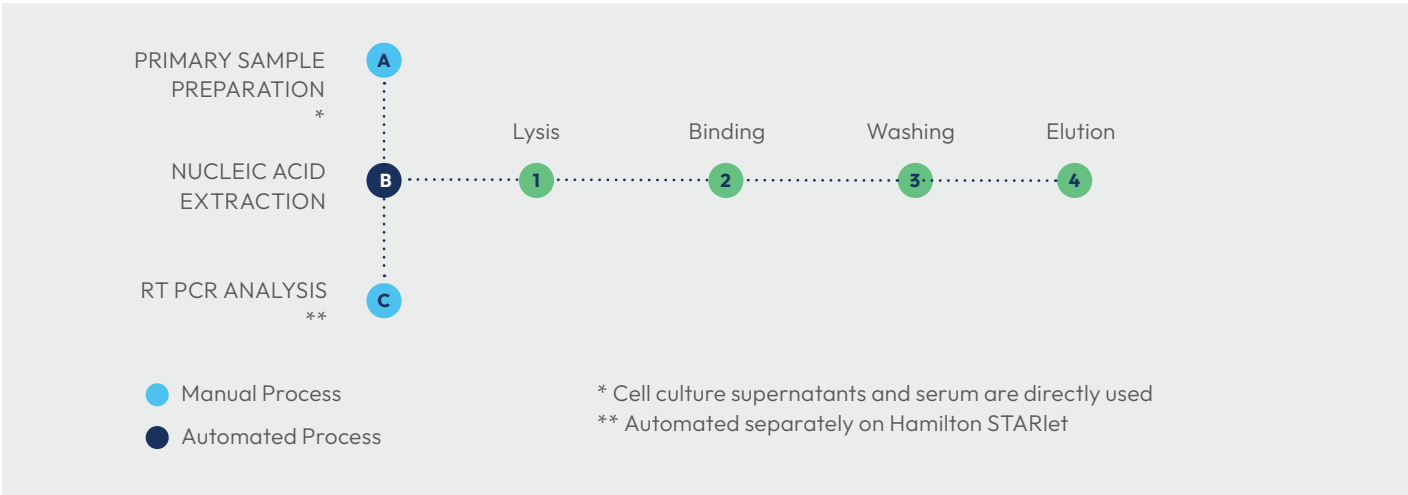


Figure 3: Visual Workflow Representation of the KYLT RNA / DNA Purification HTP Kit with Subsequent RT PCR Analysis.

Technology

The rotation stirring technology of the KYLT Purifier product line offers superior mixing performance of beads and reagents while reducing the risk of cross-contamination, due to the minimal production of aerosols and lower liquid levels. The integration of the KYLT Purifier Element on the Hamilton STARlet combines the optimal usage of lab space with exceptional

throughput and performance. The plates for the KYLT Purifier Element are transported with the CO-RE Grippers to and from the transfer position. Additionally, the method utilizes the capacitive Liquid Level Detection (cLLD) technology of Hamilton CO-RE tips.



Figure 4: The KYLT Purifier Element device integrated on the Hamilton STARlet platform. Detailed view of the integration space.

Results

In the established diagnostic workflow at the ANICON laboratory, all samples for PCR testing follow the same route after initial sample preparation, which is dependent upon sample origin. Of the resulting liquid samples, provided in primary tubes on the Hamilton STARlet, 200 μ L are used for the extraction process. Finally, clean eluates of 100 μ L RNA and DNA are produced, which are tested with one or more of the KYLT diagnostic PCR kits.

Samples originating from livestock animals, such as nose swab (swine), tracheal swab (chicken), cloacal

swab (chicken), lung tissue (pig), fecal sample (bovine, chicken, swine), sock swab (turkey), colon/liver tissue (chicken), blood sample (swine), ANIVAC vaccine and ANICARD sample were used. Resulting extracts were tested by PCR using different KYLT kits according to manufacturer specifications.

The data indicates that the results are at least as good as with the previous automation setup, but in many cases the sensitivity was increased due to improved processing (lower Ct values) using the Hamilton STARlet with integrated KYLT purifier technology.

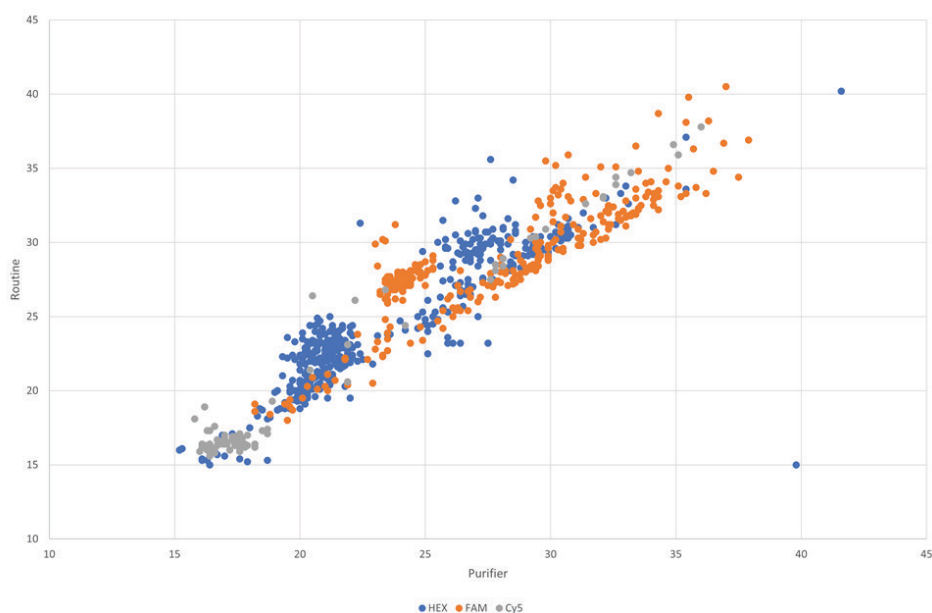


Figure 5: Dot Plot showing results across several different KYLT (RT-) qPCR kits comparing the Ct values achieved using the standard automation process (y-axis) with the new automation solution on the Hamilton STARlet with integrated KYLT Purifier Element (x-axis).

Throughput and Capacity

Compared to the previous routine solution at ANICON, a full 96 sample run is carried out in only 1 hour, instead of up to 2.5 hours.

This reduces the time to result, allowing veterinarians in the field to make faster decisions. In addition, the total possible daily throughput is more than doubled, en-

abling the ANICON laboratory to meet the highest demands, for example during local outbreaks of fast-spreading diseases.

This does not take into account the hands-on time for primary sample preparation, instrument setup, and downstream RT PCR setup and analysis.

Summary

For more than 7 years, the Veterinary PCR Unit in Germany has relied on two Hamilton STARlet robots for their automated RNA and DNA extraction needs from liquid samples as well as ANICARD samples sent in from customers around the world.

As is common with a service laboratory, the samples come in waves. To cope with peak sample numbers,

several solutions to increase throughput were considered. The best option has now been found with the integration of KYLT Purifier technology into the existing Hamilton STARlet robots. Continuing SAN Vet’s aim to simplify veterinary diagnostics applications while offering high quality, sensitivity, and reliability, is the next-generation automation solution.

Requirements		
Deck Hardware Component	Part Number / Provider	Quantity
STARlet Baseline	870102 / Hamilton	1
1 mL CORE II Channels	10140943 / Hamilton	8
KYLT Purifier Element	Available upon request / KYLT	1
Housing Extension and Integration Parts	Available upon request / Hamilton	1
Tip Rack Carrier	182085 / Hamilton	1
Deep-Well Plate Carrier	182090 / Hamilton	1
Tube Carrier 32 Samples (Set of 3)	173410 / Hamilton	2
Reagent Carrier for 3x 120 mL Troughs	813344 / Hamilton	1
Reagent Carrier for 5x 60 mL Troughs	194057 / Hamilton	1
MFX Base Plate	187001 / Hamilton	1
MFX Deep-Well Module and Brackets	188293 and 188133 / Hamilton	4

Disclaimer: The integration of the KYLT Purifier Element instrument is interfering with the minimal traverse height of the Microlab STARline pipetting arm. Therefore, using this custom setup is the sole responsibility of the customer.

Consumables	Part Number / Provider	Quantity / unit
RNA / DNA Purification HTP Kit	31575 / KYLT	4x 96 extractions
Purifier Spin Tips	31434 / KYLT	480 Tips
Purifier Plates	31435 / KYLT	20 Plates
Swab Wash Solution (optional)	31453 / KYLT	500 mL
IC-RNA (optional)	31132 / KYLT	100 reactions
Real-Time RT-PCR Detection Kit	Various / KYLT	100 reactions
0.5 mL PlateOne® 96-Deep-Well Plate	S1896-5000 / STARLAB	32 Plates
48-Well Plate*	43001-0062P / J.T. Baker	20 Plates
Filter Tips 1 mL (non-sterile)	235905 / Hamilton	3840 Tips
Filter Tips 300 µL (non-sterile)	235903 / Hamilton	5760 Tips
120 mL Troughs	194052 / Hamilton	12 Troughs
60 mL Troughs	194051 / Hamilton	28 Troughs

* used for processing of ANICARD samples

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