

CHRONECT Symbiosis RIDA®CREST

Automation for mycotoxin analysis





Improved reproducibility

- High precision
- Automation reducing handling errors
- Harmonisation and standardisation of methods



Improved quality

- Confidence in results first time round
- Multi-use cartridge with the option for QC checks
- Affinity clean-up improving sensitivity



More information:

<u>https://r-b.io/ridacrest</u>



04/2022

Improved productivity

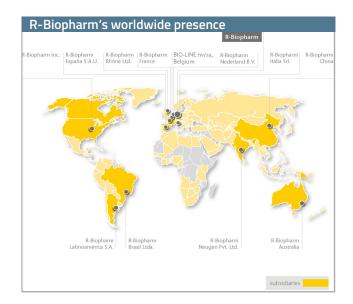
- Reduction in bench time
- Emergency samples can be added mid-run

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Global excellence in food and feed analysis

The R-Biopharm Group is a leading developer of test solutions for food and feed analysis offering products for various applications. Food processing companies throughout the world appreciate the reliable and user-friendly solutions. The company is constantly working on the development of new innovative technologies. Methods include ELISAs, real-time PCR, lateral flow tests, culture medium plates, solid phase extraction columns, immunoaffinity columns and cartridges.

- 30 years of experience in developing and manufacturing immunoaffinity products for the food and feed industry
- Excellence in research and development of new and innovative products
- Global technical support and training
- R-Biopharm products have been used in collaborative trials and referenced in Official Methods (AOAC and CEN) thereby conforming to strict method performance requirements
- Complete and cost-effective portfolio which ensures confidence in results first time round using recommended applications



Combining application expertise with technical skill



To meet the changing needs of users, R-Biopharm have partnered with Axel Semrau, a global technology partner experienced in providing advanced laboratory

solutions worldwide. This new partnership combines specialised application expertise with state of the art equipment.

For more than 35 years Axel Semrau GmbH & Co. KG has experience in the distribution and development of system solutions for chromatography and mass spectrometry. One particular focus is the efficient automation of complex work flows in the laboratory. Axel Semrau have developed their own software to assist with the sample preparation and analysis which enables a time efficient design of the laboratories work flow. The automation solutions are sold under the brand name CHRONECT and the self developed control software is named CHRONOS.

CHRONECT Symbiosis couples automatic sample preparation with effective online clean-up. The solutions are distributed by dealers all over the world. The devices and solutions are used in research and development, quality control and analysis. In the field, Axel Semaru are known for innovative and reliable solutions.



The future is automation

Numerous high-throughput food and feed companies are moving towards automated solutions to improve work flow and increase productivity.

Many global companies are also interested in harmonising and standardising methods within and between different sites to enable improved sharing and transfer of methods and data in an effort to increase efficiency. R-Biopharm have developed a range of unique and innovative immunoaffinity cartridges, IMMUNOPREP[®] ONLINE, for mycotoxins and vitamins, which are automated via the CHRONECT Symbiosis RIDA[®]CREST system.

Market factors influencing change to automation

- Time pressure on laboratories is increasing
- Demands to increase throughput
- Demands to decrease overheads
- Staff costs are increasing

- Improve quality of results to meet ISO 17025 requirements
- Increased regulations and safety standards
- Standardisation and harmonise methods between sites
- Bring testing in-house

automation presents an opportunity to manage these factors

R-Biopharm product portfolio

Immunoaffinity columns	Immunoaffinity cartridges	Automated system
 Contains monoclonal antibodies for mycotoxins Specific clean-up removing sample interference Concentrates analyte to improve sensitivity Multi-toxin products which can improve and optimise workflow Single use products 	 Contains monoclonal antibodies for mycotoxins Specific clean-up removing sample interference Concentrates analyte to improve sensitivity Reusable Option to Quality Control each cartridge 	 Automated handling system for working with IMMUNOPREP® ONLINE immunoaffinity cartridges Processes two cartridges simultaneously saving time Standardised method leading to improved accuracy Automated clean-up resulting in faster turnaround time and greater efficiency Pre-programmed applications for a variety of matrices Platform technology with new products in development, including cartridges for vitamins

Automated immunoaffinity clean-up

The equipment: CHRONECT Symbiosis RIDA®CREST

- The IMMUNOPREP® ONLINE cartridges are used in combination with the CHRONECT Symbiosis RIDA®CREST system
- CHRONECT Symbiosis RIDA[®]CREST is a UHPLC system for connection to either a fluorescence, UV or MS detector
- The CHRONECT Symbiosis RIDA[®]CREST system contains two modules (ACE and HPD) for handling and processing the cartridges
- Flexibility to use as a general UHPLC for other analytes and for analysis of eluates following offline immunoaffinity column clean-up

Module	Specification
1. Gradient pump	SPH1299 UHPLC pump
2. Autosampler	CTC-PAL LSI
3. HPD (High Pressure Dispenser)	1x syringe, 1x valve, 2x SSH for mixing
4. ACE (Automated Cartridge Exchanger)	Dual clamp, 4x valve (UHPLC)
5. Column oven	Mistral cool (5 - 90 °C)
Software	CHRONOS





CHRONECT Symbiosis RIDA®CREST products and specifications

Module	Specification	Features	Benefits
Gradient pump	SPH1299 (UHPLC) pump	 Eliminates need to sonicate and de-gas mobile phase Automatic purge and prime functions Automatic compressibility compensation Consistent pump flow 	 Reduced preparation time Accurate pump performance Reduced pressure fluctuations Reduced baseline noise Improved detection limits
Autosampler	CTC-PAL LSI	 Individual injection ports for direct and IMMUNOPREP® ONLINE injections Ability to change sample loops Large sample vial capacity Additional modules can be purchased to allow sample filtration, centrifugation, dilution and mixing 	 Flexibility to fit with current laboratory methods and work flow
High pressure dispenser (HPD) module	1x syringe, 1x valve, 2x SSH for mixing	Controled flow rateAccurate loading volumes	High precisionConsistent results
Automated cartridge exchange (ACE) module	Dual clamp, 4x valve (UHPLC)	 Tubing configuration identifies each valve with a specific function Integrated waste lines Small internal diameter tubing 	 Easy to troubleshoot Sharp peaks and better resolution Improved detection limits
Column oven	Mistral cool (5 - 90 °C)	 Additional options available Factory upgrade possible to allow column switching for up to 6 analytical columns 	 Compatabile with FLD, UV and MS detectors
Software	CHRONOS & Clarity™	 Direct control of CHRONECT Symbiosis RIDA®CREST hardware Allows acquisition of multiple sample sequences Two-cartridge IMMUNOPREP® ONLINE model developed using one method making it easy to learn software routines and amend sequences. Links to various data acquisition packages such as Clarity[™], Analyst, and MassLynx 	 Compatible with own software More flexible for sample and data processing

Product code	Standard configuration	Product code	Available upgrades
RIDACREST AS-WS-0511	 SPH pumps 1299 CTC-PAL-LSI autosampler ACE 		 Mistral cool CS (6 analytical column Peltier cooling trays Barcode reader
	HPDCHRONOS software		

IMMUNOPREP[®] ONLINE products

Mistral cool column oven

	Art No.	Product	Format	No. of tests
	Online mycotoxin immunoaffinity cartridges			
	P900/48, P900	IMMUNOPREP® ONLINE AFLATOXIN	re-usable cartridge	48 / 96
	P904/48	IMMUNOPREP® ONLINE AFLATOXIN M1	re-usable cartridge	48
	P901/48, P901	IMMUNOPREP® ONLINE OCHRATOXIN	re-usable cartridge	48 / 96
	P902/48	IMMUNOPREP® ONLINE DEOXYNIVALENOL	re-usable cartridge	48
	P903/48	IMMUNOPREP® ONLINE ZEARALENONE	re-usable cartridge	48
COMING SOON!	P905/48	IMMUNOPREP® ONLINE FUMONISIN	re-usable cartridge	48
	Online vitamin immunoaffinity cartridges			
COMING SOON!	P800/48, P800	IMMUNOPREP® ONLINE VITAMIN B12	re-usable cartridge	48 / 96

IMMUNOPREP® ONLINE description

- The monoclonal antibody is bound to a rigid hydrophilic polymer, which is packed into the small cartridge
- Automation and use of the IMMUNOPREP[®] ONLINE cartridge is performed using the CHRONECT Symbiosis RIDA[®]CREST system



The disposable: IMMUNOPREP® ONLINE cartridges

- A patented technology
- Monoclonal antibody bound to a rigid polymer
- Can withstand high pressures of HPLC or LC-MS/MS
- Cartridges are processed and samples are analysed directly online
- Combines benefits of immunaoffinity clean-up with automation



IMMUNOPREP® ONLINE cartridges are reusable up to 15 injections

- Two cartridges analysed simultaneously, providing analytical results faster
- Standards can be injected through the cartridge to monitor the analytical run enhancing Quality Assurance as per ISO 17025
- Enviornmental benefits due to reduced packaging and chemicals
- Opportunity for improving ergonomics and productivity

Improved reproducibility

- High precision
 - Automation removes variability

Improved productivity

• Reduction in bench time

Improved performance

• Pre-programmed, optimised methods

Robust methods

• Confidence in results first time around

Improved quality

 Standard or blank can be loaded between sets



Comparision of immunoaffinity column and cartridge clean-up





1. Booking in



2. Weigh sample



3. Extraction



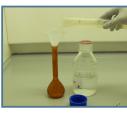


mmunoaffinity column work flow

6. Analysis



7. Results



4. Filtration

Clean-up with IMMUNOPREP® ONLINE cartridges provides -

Benefits of immunoaffinity clean-up

- High selectivity
- Concentration of analyte from sample matrix
- Removal of interfering components

Combined with benefits of automation

- Time and labour savings throughout process
- Increased capacity
- Improved traceability and reproducibility
- Improved Quality Control



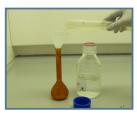
1. Booking in



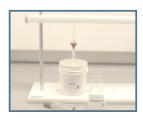
2. Weigh sample



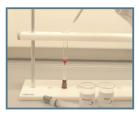
3. Extraction



4. Filtration



5. Applied to IAC



6. Wash & elute



7. Transfer filtrate



8. Results



Sample procedure prior to automated analysis



Extract sample and filter.



Dilute sample, transfer to autosampler vial and insert into autosampler.

Clean-up and analysis on cartridge is fully automated.

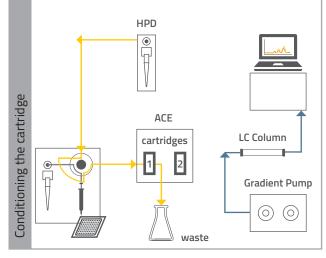
Two cartridges are run simultaneously reducing time to obtain results. A maximum of 15 samples are analysed with

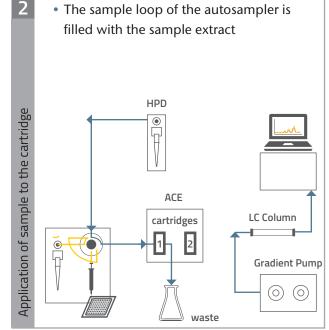
each cartridge.

What's happening in the CHRONECT Symbiosis RIDA®CREST system?

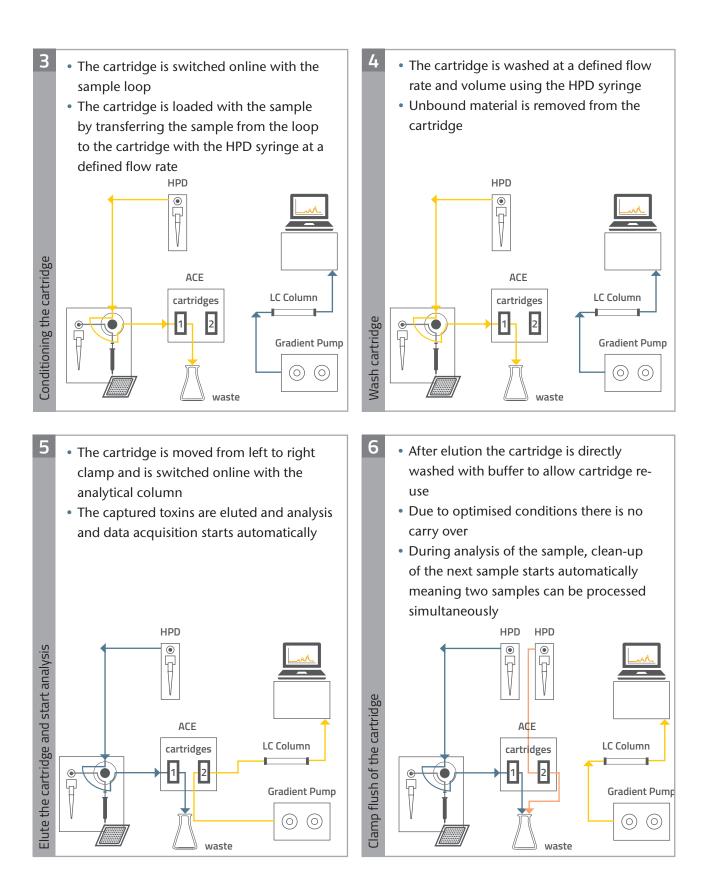


- The cartridge is removed from the tray and placed in the left-hand clamp of the ACE
 - Loading buffer is passed over the cartridge to condition it before sample loading



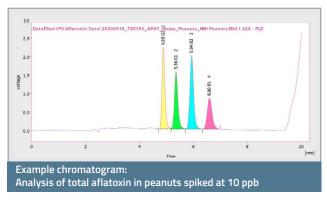


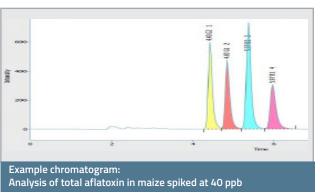


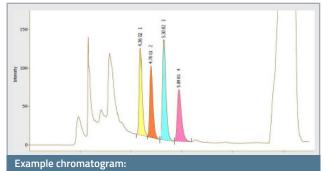


Performance data for aflatoxin analysis

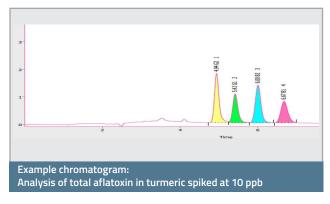
Sample	Spike level (ppb)	% Recovery (% RSD)			
		AFT B1	AFT B2	AFT G1	AFT G2
Maize	5	95.1 (0.6)	95.5 (0.4)	95.4 (0.5)	97.3 (1.1)
Peanuts	10	78.8 (0.3)	82.4 (0.2)	86.4 (0.3)	88.3 (0.3)
Cumin	0.5	87.8 (2.1)	89.4 (1.3)	91.0 (2.5)	95.1 (0.8)
	2.5	87.3 (1.0)	87.3 (0.5)	89.2 (0.3)	90.8 (1.3)
Chilli	0.5	-	91.1 (1.2)	92.8 (1.1)	94.7 (1.8)
	2.5	104.0 (0.8)	83.7 (0.2)	84.9 (0.3)	84.1 (0.8)
Coriander	0.5	91.7 (1.0)	93.5 (0.9)	94.3 (1.4)	95.7 (2.0)
	2.5	87.5 (0.7)	87.2 (0.8)	88.1 (0.6)	89.3 (0.9)
Turmeric	0.5	83.1 (3.7)	87.7 (2.0)	90.6 (1.2)	92.6 (1.1)
	2.5	79.1 (0.9)	81.0 (0.1)	82.1 (0.2)	87.2 (0.8)
Ginger	0.5	104.5 (2.0)	90.4 (0.6)	92.6 (0.8)	92.3 (1.1)
	2.5	96.0 (1.8)	93.2 (1.6)	90.7 (1.4)	89.7 (2.8)
Black pepper	0.5	82.6 (3.3)	82.4 (3.9)	78.6 (1.3)	76.7 (0.7)
	2.5	90.6 (1.2)	90.6 (1.2)	86.6 (0.6)	86.1 (0.7)







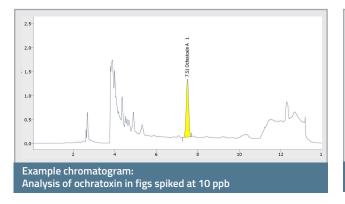


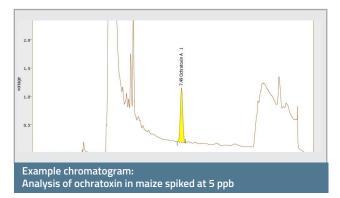


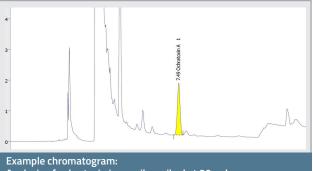


Performance data for ochratoxin analysis

Sample	Spike level (ppb)	% Recovery (% RSD)
		ОТА
Figs	5	101.3 (1.7)
	10	103.1 (1.8)
	20	93.2 (0.4)
Paprika	10	76.3 (6.8)
	20	87.4 (4.8)
	40	71.6 (0.8)
Wheat	2.5	86.5 (1.2)
	5	95.1 (0.9)
	10	80.0 (5.2)







Analysis of ochratoxin in paprika spiked at 20 ppb

CHRONECT Symbiosis RIDA®CREST with IMMUNOPREP® ONLINE

Combined benefits

- Re-usable immunoaffinity cartridges
- Pre-programmed applications for a variety of analytes
- Automated cartridge clean-up and concentration of analyte, suitable for testing complex matrices
- Flexible equipment allows automation of direct injection for clean samples
- An automated platform for a growing number of analytes

Improved laboratory work flow

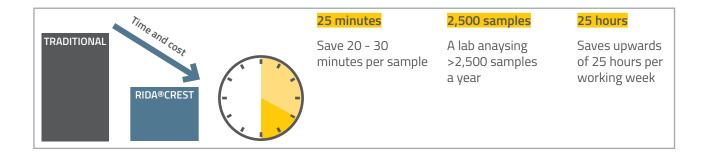
- Two cartridges are run simultaneously, reducing analytical time and increasing throughput
- Automation of the cartridge application online reduces handling time at the bench
- Flexible programs allow emergency samples to be added mid-run

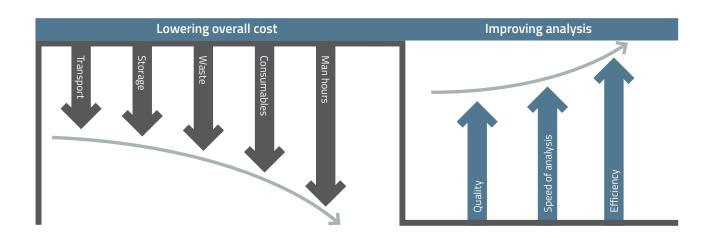
Enhanced quality performance

- Unique, automated device that can include online quality control
- Reproducible and accurate results used in combination with your chosen detector
- Monoclonal antibodies improve selectivity and sensitivity resulting in better limits of detection and quantification
- Exceeds CEN and AOAC Method Performance Criteria for mycotoxins
- Ensuring the best results are achieved first time around

Eco-friendly

- Less solvents used and discarded to waste
- A re-usable product means less storage and transport costs
- A mini cartridge means less plastic and waste







Who would benefit from automation?

- Laboratories testing >2000 samples per annum
- Laboratories planning to switch to automation
- LC-MS/MS users with complex samples to analyse

automation presents an opportunity to manage these factors

Many laboratories are interested in automated systems for a variety of reasons and there are numerous factors to consider before investing. R-Biopharm offers a consultative service to assist in selecting the most appropriate product to optimise work flow saving time and money. The following are examples of cases studies for labs considering automation for the quantitative analysis of mycotoxins.

Laboratories wanting to increase sample throughput

Laboratories wishing to bring testing in-house

Dairy company

"We are a company who are predominantly testing dairy products and analysing aflatoxin M1 in the range of 0–0.5 ppb. We also analyse certain samples for vitamin B12.

For both analytes we previously used immunoaffinty columns for sample clean-up however sample numbers were increasing and we were analysing 50 -70 samples per week. Our aim was to reduce manual clean-up and time spent at the bench as well as trying to reduce errors which occured during the manual processing of samples.

We have now installed the CHRONECT Symbiosis RIDA®CREST system alongside our FLD-UHPLC detector and have been using the system routinely for a number of months. We found the onsite installation quick and easy and our methods were preprogrammed which meant rapid implemenation of the system.

We were immediately able to benefit from quicker results which meant we could release product earlier. In addition, we were able to reduce the bench time and improve our throughput without compromising on the quality of results.

We are so impressed with the system and are eagerly waiting for the launch of the new IMMUNOPREP[®] ONLINE VITAMIN B12 cartridges so that we can convert to automated analysis for this analyte."



- IMMUNOPREP[®] ONLINE AFLATOXIN M1 (Art. No.: P904)
- EASI-EXTRACT[®] VITAMIN B12 (Art. No.: P80)
- IMMUNOPREP[®] ONLINE VITAMIN B12 following product launch (Art. No.: P800)

Government laboratory

"We are responsible for ensuring that food produced and sold in the country complies with regulations. We therefore work with a wide range of commodities, many of which are finished products and can be challenging to analyse. We therefore use a range of immunoaffinity columns which we purchased from an alternative supplier.

However, we were looking at ways to save time and increase throughput in order to transfer the labour and resources saved to other analysis. It was important to maintain high quality, repeatable results for mycotoxins with the range of samples tested and to ensure that we could meet legislative criteria.

We had an LC-MS/MS system in-house therefore initially considered multi-toxin analysis. R-Biopharm were very supportive and provided a range of options to help us select the appropriate products to meet our objectives. In the end we selected the CHRONECT Symbiosis RIDA®CREST system with IMMUNOPREP® ONLINE cartridges for automated analysis of aflatoxin and ochratoxin in conjunction with our LC-MS/MS detector as this accounted for the majority of our analysis (around 70 % of 2,500 analysis). For the remaining analytes we implemented a multi-toxin approach.

This combination allowed us to save time and labour. In fact, we have estimated that we save approximately 25 minutes/sample when using the CHRONECT Symbiosis RIDA®CREST system which equates to 25 hours/month. This has improved our efficiency and productivity.

The technical team at R-Biopharm worked closely with us during the installation period which meant that there was minimum change to in-house extraction methods and the IMMUNOPREP® ONLINE cartridges could be used with Official Methods. The CHRONECT Symbiosis RIDA®CREST system was able to process eluates from immunoaffinty columns for other mycotoxins using a direct injection port."



- IMMUNOPREP[®] ONLINE AFLATOXIN (Art. No.: P900)
- IMMUNOPREP[®] ONLINE OCHRATOXIN (Art. No.: P901)
- DZT MS-PREP®
- (Art. No.: P73) • FUMONIPREP[®] (Art. No.: P31)



Private laboratory

"Our company has world class testing capabilities therefore it is important that our labs located globally have standardised methods between sites to ensure compliance with regulations.

In many sites, lab space and resources are limited and the number of mycotoxin requests are increasing. We currently analyse using a mixture of direct injection and immunoaffinty clean-up however it is difficult to increase sample numbers further without -

- increasing headcount
- compromising on test performance
- compromising on quality of results.

At this site, we currently analyse 5,000 cereal samples and 3,000 complex samples for aflatoxin and ochratoxin A per year. In addition, 2,000 cereal samples for either deoxynivalenol or multiple mycotoxins are analysed. LC-MS/MS was our preferred detection method as cereals can be analysed using a less expensive QuEChERS method without the need for affinity clean-up.

We contacted R-Biopharm to find out how the CHRONECT Symbiosis RIDA®CREST system could work for us. The implementation of the affinity clean-up removed the need to use isotopic labelled and matrix matched standards for complex samples which saved time and money. In addition, it provided a better clean-up which reduced downtime and maintenance costs for our LC-MS/MS. Using a combination of immunoaffinity columns and affinity cartridges allowed harmonisation of methods within and between sites, improving consistency in results due to automation."



- IMMUNOPREP[®] ONLINE AFLATOXIN (Art. No.: P900)
- IMMUNOPREP[®] ONLINE OCHRATOXIN (Art. No.: P901)
- DONPREP[®] (Art. No.: P50)
- 11⁺Myco MS-PREP[®]
 - , (Art. No.: P128)

Company that currently outsources testing

"We previously only conducted small numbers of vitamin analysis in-house using immunoaffinity columns despite having our own in-house laboratory and trained technical staff. The majority of samples were sent to an external lab for analysis - particularly for mycotoxins.

Costs for the external analysis were increasing therefore we wanted to bring aflatoxin M1 testing inhouse to reduce overheads and to improve the speed of results. We currently had to wait 4 - 6 working days. We routinely sent 25 samples per week for analysis with a cost of \geq 100 per test for aflatoxin M1.

It was important that the method we implemented was suitable for a range of matrices including butter, cream and whey and must meet legislative requirements. Performance and sensitivity of the test was also important.

Using the CHRONECT Symbiosis RIDA®CREST and IMMUNOPREP® ONLINE AFLATOXIN M1 cartridges we have been able to significantly reduce the annual spend on outsourcing and have been able to provide results to our customers quicker. We were able to easily reach required sensitivity to comply with aflatoxin M1 legislation. Overall, we have been impressed that testing samples in-house is more cost effective, with the return on investment for the CHRONECT Symbiosis RIDA®CREST system being achieved within a short time frame of around 18 months."



- IMMUNOPREP[®] ONLINE AFLATOXIN M1 (Art. No.: P904)
- EASI-EXTRACT[®] VITAMIN B12 (Art. No.: P80)
- IMMUNOPREP[®] ONLINE VITAMIN B12 following product launch (Art. No.: P800)



CHRONECT Symbiosis RIDA®CREST

Automation for mycotoxin analysis

Dedicated support service

- Installation of CHRONECT Symbiosis RIDA[®]CREST handling system
- Assistance with on-site method validation
- Provision of in-house training on equipment and associated software
- Consultative service to assist in selecting the most appropriate product
- Provision of bespoke applications
- Technical support and advice through a dedicated channel

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