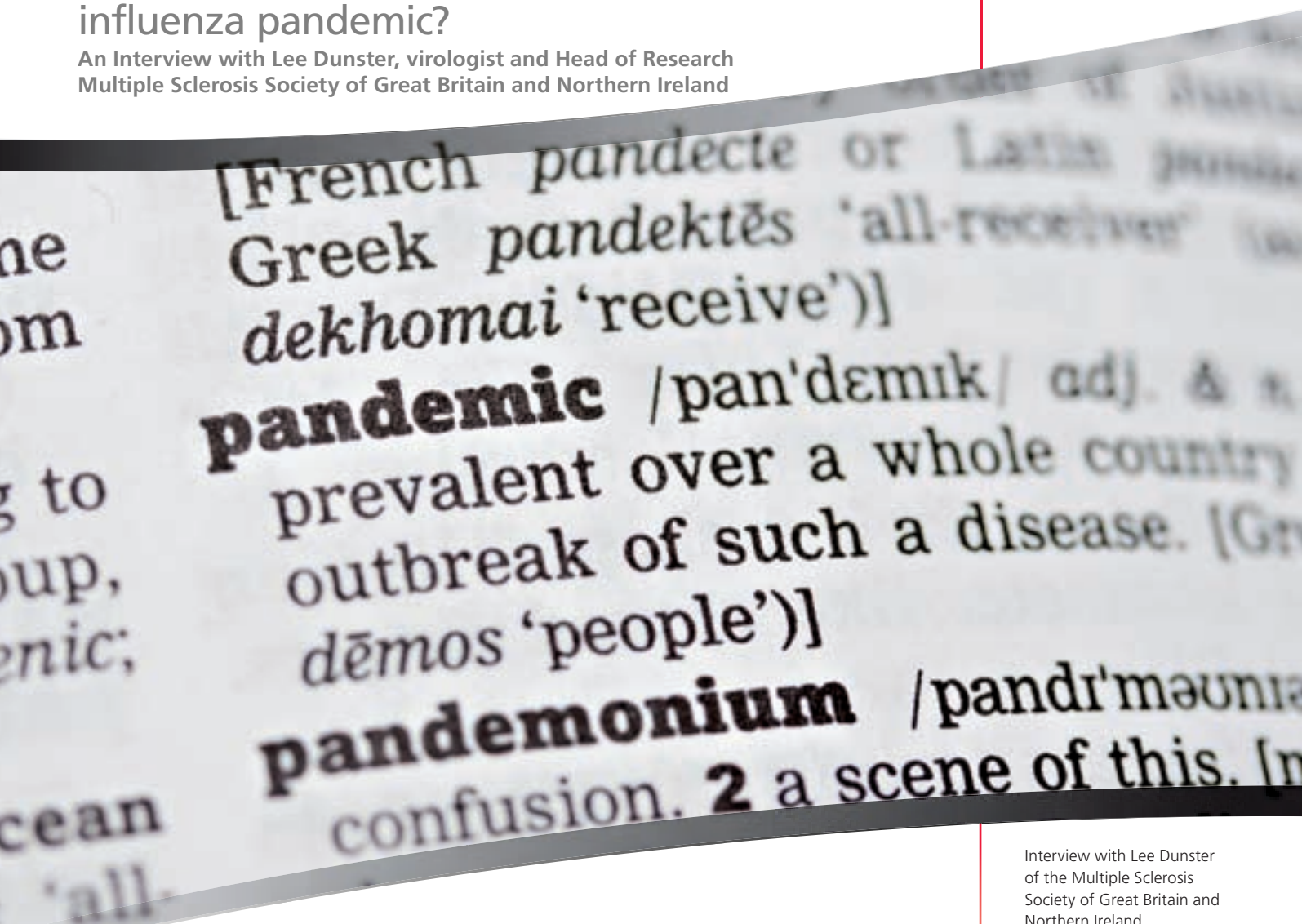


# INVITRO

Issue 5 • 2009

## What can we learn from the current influenza pandemic?

An Interview with Lee Dunster, virologist and Head of Research Multiple Sclerosis Society of Great Britain and Northern Ireland



Interview with Lee Dunster  
of the Multiple Sclerosis  
Society of Great Britain and  
Northern Ireland

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Fungi in Human Tissue

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Our Innovation, Your Research – Shaping the Future of Life Science

## Dear Researcher,



Sigma-Aldrich Chemie GmbH  
Walter Gmelin, PhD

It is our great pleasure to present an interview with Lee Dunster, Head of Research, Multiple Sclerosis Society of Great Britain and Northern Ireland.

Lee Dunster is a vibrant member of our community with an extensive track record in the research of virology, leaving the lab to create and implement new and innovative strategies for large research programmes. As his curriculum vitae and this interview demonstrate, though our field of interest can change over time, we can continue to contribute to the advancement of science, whether it is through basic research or, in this case, by establishing clear strategies for research programmes.

Pathogens are always key topics of our BioFiles In Vitro journal and in our scientific article for this issue, we present a fast, highly specific histological detection of pathogenic fungi in human tissue based on our fluorescently labelled lectins. This article is the result of a close research collaboration between our R&D department and the renowned Inselspital University Hospital in Bern, Switzerland.

This edition also contains interesting new articles on our Prestige Antibody® range, to which we have added another 2,300 antibodies over the past 6 months. The benefit of these antibodies can be truly seen in the comprehensive validation data being provided by the Human Protein Atlas database. We will continue to report in future issues of BioFiles In Vitro about Prestige Antibodies and provide you with an update on the current status of new antibodies available.

We continue with our regular series on classical histological stain protocols. In this issue, we have included two protocols based on protocols we provide in accordance with the IVD product range, which can be collected in your lab work.

You will find a scientific article written in collaboration with Caprion® on the "Development of a Fast and Simple One-Dimensional Separation Approach for the Detection of Low Abundance Plasma Proteins". In this article, we present an easy method using our SuperMix depletion columns to detect low abundance protein for LC/MS analysis.

Wrapping up this issue is an article about "detecting disease carrying ticks using a new microbiological rapid test system". Read about the fast and reliable detection of Lyme borreliosis using Scanbec® technology, which has been validated in collaborative studies.

Finally, we hope that you enjoy reading our BioFiles In Vitro journal.

Kind regards,

Walter Gmelin, PhD  
European Marketing Manager Life Science

P.S.: We encourage you to present your work in the form of an article or interview in future issues. Please feel free to contact us.

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# Interview with Lee Dunster

Virologist and Head of Research, Multiple Sclerosis Society of Great Britain and Northern Ireland

## CURRICULUM VITAE

### Career:

<b>March 2003 – Current</b>	Head of Research Multiple Sclerosis Society of Great Britain and Northern Ireland
<b>Jun 2002 – Oct 2002</b>	Consultant Scientist, World Health Organisation (WHO), Duty station: WHO Regional Office, Cairo, Egypt
<b>1996 – 2002</b>	Chief of Operations, WHO, Collaborating Centre for Arboviruses and Viral Haemorrhagic Fevers Reference and Research, Nairobi, Kenya
<b>1991 – 1996</b>	Alexander von Humboldt Post-doctoral Research Fellow, Institute for Virology and Immunology, Germany



### Qualifications/education:

- PhD, Department of Microbiology, University of Surrey, UK, 1990.  
Thesis: The attenuation of flaviviruses following passage in HeLa cells.
- BSc (Hons), 1st in Microbiology, University of Surrey, UK, 1987.

Following his PhD in virology in the UK and becoming a key member of a world-class research group in Germany, the British scientist Lee Dunster left the lab to create and implement new and innovative strategies for large research programmes. From 1996–2002 he worked as Chief of Operations for the WHO Collaborating Centre for Arboviruses and Viral Haemorrhagic Fevers Reference and Research in Nairobi, and as a Consultant Scientist for the WHO in Egypt. In March 2003, he returned to the UK and started to implement a new strategic direction for the MS Society, the world's third largest charitable funding organisation of research into the cause, cure and care of multiple sclerosis.

**IN VITRO: Dr. Dunster, you recovered recently from the swine flu, so you're a virologist who turned into a person concerned.**

**LD:** It's a bit like saying hello to an old friend having worked on the use of the drug amantadine to control influenza outbreaks in the mid 80's, which is what oseltamivir, Tamiflu®, is designed for today. The virus still put me in bed for a few days, which gave me a chance to catch up on some outstanding work on the laptop.

**IN VITRO: Talking about another virus – what did it feel like during your time in Africa when Ebola was near?**

**LD:** I guess the first feeling was 'this is it; this is what we're here for'. When the



outbreak took hold in Uganda, I'd been in Kenya for over four years building the diagnostic, surveillance and response capacities for the arboviruses and viral haemorrhagic fevers. This was an opportunity, albeit a serious one, to test the system we had in place.

**IN VITRO: As leader of the national programme to prevent the disease entering the country from neighbouring Uganda, you must have been under big pressure ...**

**LD:** Truth be told, this kept me awake at night. I had been handed responsibility by the Kenyan Ministry of Health, which ultimately meant that I would be the scapegoat if this went pear-shaped and we had an outbreak. The responsibility, pressure and expectations were quite incredible. Perhaps the greatest concern was the open and very porous nature of the border between Uganda and Kenya. On top of this was my big fear that an infected individual could make it to a large city like Nairobi and then we could have real problems.

**IN VITRO: What was your strategy for prevention?**

**LD:** First and foremost, we needed to raise public awareness and education – to dispel the myths that were flying around – and this is where the national media was very useful. We also had specific education sessions for clinicians on what to look out for and how to instigate isolation and barrier nursing procedures until diagnostics could be completed. The key thing was to recruit the help of the local villages and towns that ran the length of the border – no matter how remote.

**IN VITRO: How could they have helped?**

**LD:** The village chiefs and town leaders were to be the eyes and ears for the surveillance programme as they know their communities and could easily spot 'strangers', or keep



track of people recently returned from Uganda for the appearance of unexplained symptoms and then alert the authorities. As you'd expect, this put a strain on the resources and, in particular, on my small team who were suddenly receiving calls of unexplained fever with bleeding, which given the circumstances, needed to be assessed as local healthcare professionals were often reluctant to get involved initially. This changed with the provision of training and education in the use of barrier procedures for investigation of suspect cases and the all important collection of samples for testing back at the lab in Nairobi.

**IN VITRO: How can a quick and reliable diagnosis be guaranteed in remote regions at the place of the outbreak?**

**LD:** The key is getting the samples to the WHO lab as speedily as possible and then having the capacity to test them fast and accurately. Even from remote locations, we would typically have specimens within 48–96 hours from a surveillance site. Much shorter if we travelled there ourselves – perhaps 24–48 hours. With a huge amount of international support, the lab in Kenya went from having a very limited capacity on my arrival, to being able to diagnose a range of



arboviruses and viral haemorrhagic fevers endemic to the East Africa region. When I left Kenya in 2002, the laboratory had capacity for virus isolation in basic Category 3 safety facilities built by local hands, serology and RT-PCR, which was unique in East Africa at that time. The lab in Nairobi had proved to be sustainable and a first-class diagnostic facility for the region – five years of hard work had paid off. That was great.

**IN VITRO: What can be learned from your experience in Africa for containment of the current pandemic influenza disease?**

**LD:** I'd say the crucial thing is to have good ground surveillance in place and effective communication channels. Any situation can get rapidly out of control if communication is poor. What I mean is that communication between national and international authorities needs to be open so that they can share experiences, best practices and cooperate with each other. Above all, you must provide the public with clear up-to-date information that is open and honest but not alarmist. If the public has all the relevant information, then a significant part of the battle is won.

**IN VITRO: Why could Ebola outbreaks be locally controlled in the past, but this has been never achieved for influenza?**

**LD:** This is due to differences in how the viruses are transmitted. Ebola is transmitted through close contact with infected body fluids. Influenza is rather different in that it is airborne and can be dispersed rapidly and easily to many contacts. The best bet with pandemic influenza is the vaccine approach, but as we have seen in this current outbreak, there is a significant time delay between identification, isolation, production, testing and distribution of a new vaccine. We are fortunate that the current H1N1 swine flu is presenting itself as a mild illness overall.

**IN VITRO: So H5N1 years before was of major concern?**

**LD:** This flu had alarming mortality rates but was very poor at transmitting from human to human. The H5N1 virus is being closely monitored by the World Health Organization (WHO) as a significant potential threat. On the basis of this, the WHO has worked tirelessly with governments throughout the world to establish flu surveillance and improve national influenza outbreak preparedness and response strategies. I strongly believe that the current H1N1 pandemic is testing these systems in preparation for a more virulent influenza virus of the future – and that is a good thing.



**IN VITRO: In your career and as the scientific head of the MS society, you have demonstrated an ability to improve research programmes. What are your major achievements?**

**LD:** The key thing was to develop a clear research strategy to deliver outcomes for people affected by MS within the operating constraints I was given. This required a complete overhaul, driving through major changes to a more innovative evidence-based research programme. This has gone hand-in-hand with a complete revision of the infrastructure required to deliver the strategy – I am talking here of manpower, skills and the expertise needed to achieve results. The Society now has a burgeoning and successful MS care and services research programme. Just recently, the programme won the 2009 Association of Medical Research Charities Award for Public and Patient Involvement. I'm really proud of my achievements at the MS Society over the past six years, but I now feel that the time has come to move on and search for my next challenge.

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# Fast and Highly Specific Histological Detection of Pathogenic Fungi in Human Tissue

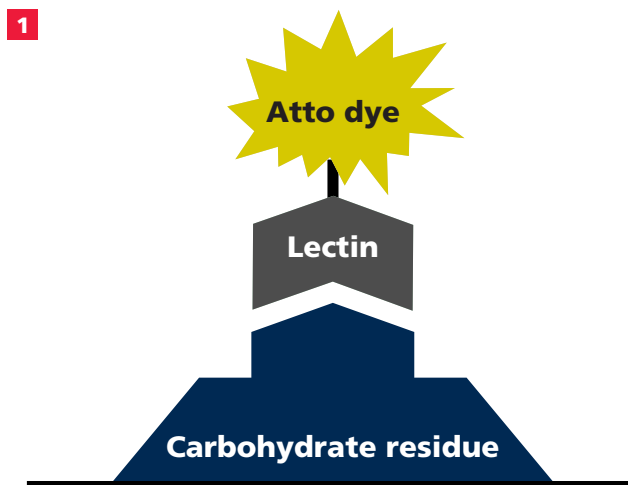
Monika Baeumle, PhD, Product Manager Biochemistry, monika.baeumle@sial.com  
 Bernhard Schoenenberger, PhD, Supervisor R&D  
 Thomas Milesi, R&D, Scientist, Associate  
 Jakob Zbaeren, Thrombose Laboratory, Inselspital Bern

Lectins are ubiquitous proteins or glycoproteins that can be isolated from plant and animal sources, and can bind to specific carbohydrate moieties. Due to their high affinity to sugar residues, lectins have become important tools for sensitive detection of cellular carbohydrates, revealing subtle alteration in glycosylation between otherwise indistinguishable cells. This allows identification of cellular surface structures, e.g. cell surface, cytoplasm, and nuclear structures. Furthermore, lectin affinity binding allows for the detection of pathogenic degeneration of tissue as well as pathogenic infestations such as fungi.

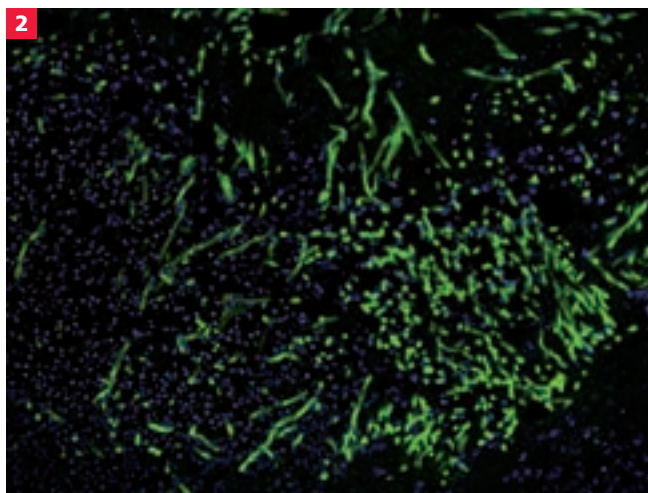
Histochemical studies are of importance in the histological and pathological investigation of tissue in clinical research. Lectin histochemistry can be performed on living cells in suspension, on cell smears, tissue imprints, fixed tissue sections or fresh cryostat sections.

The recently developed Atto-dye labelled lectins have many applications, including carbohydrate, mitogenic and histochemical studies. Atto-dyes have very bright fluorescent signals and high photo stability, which enable a direct one-step tissue-binding protocol. Time-consuming multistage amplification procedures are not required for Atto-dye lectin conjugates. Here, we demonstrate a highly specific identification of pathogenic fungi on human tissue via direct fluorescence detection using fluorescently labelled lectin (**Figure 1**).

Lectin histology was performed on both polymer and paraffin-embedded human skin tissue. The lectin conjugate used was *Phytolacca americana*-Atto 488 (**Cat. No. 39905**). The conjugate was diluted 100x in PBS buffer (pH 7.4) before incubating with each specimen for 30 min. After washing to remove any unbound lectin and counterstaining the nuclei with DAPI (**Cat. No. 32670**), the samples were examined using a microscope equipped for epifluorescence with a 450–490 nm excitation bandpass filter and a 520–560 nm barrier (emission) filter.



**Figure 1:** Direct one-step binding of fluorescently labelled lectins.



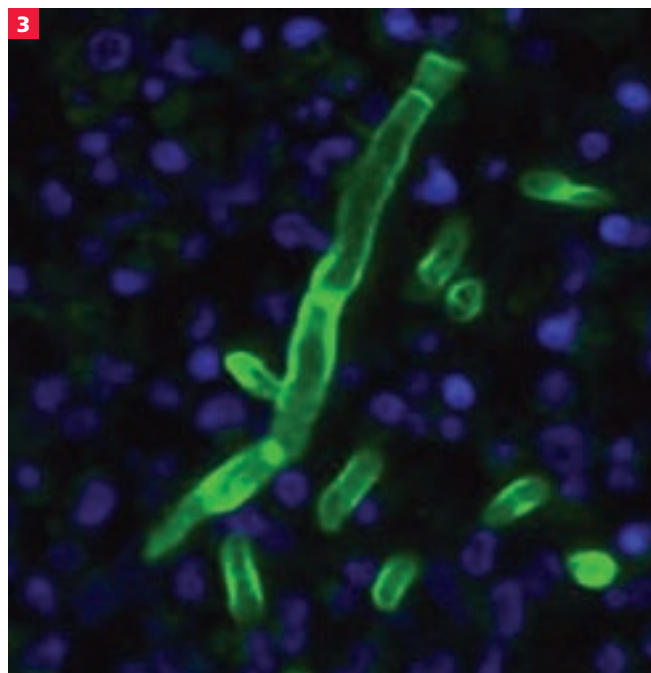
**Figure 2** Fluorescence microscopy of a human skin tissue section (paraffin fixation) with fungal infection. The target carbohydrate subunit chitotriose [(GlcNAc)<sub>3</sub>] of the pathogenic fungi are specifically bound to lectin from *Phytolacca americana*-Atto 488 conjugate (green). The nuclei are counterstained with DAPI (blue).

Image by J. Zbaeren, Inselspital Bern, Switzerland.

The images obtained show a very specific labelling of pathogenic fungi infecting human tissue (**Figure 2**). The image demonstrates the fine filaments of the fungi containing typical mycelium, and individual fungi cells are clearly visible (**Figure 3**). A slightly higher fluorescence is observed in the separating cross-walls between two cells (septa), which is due to a higher concentration of target carbohydrates. Very low background is observed.

Fungal cell walls contain chitin, a polymer of  $\beta$ -(1 $\rightarrow$ 4) linked N-acetyl-D-glucosamine, while animal and plant cells do not synthesise chitin. The lectin *Phytolacca americana* targets the fungal carbohydrate fragment chitotriose [ $(\beta$ -N-Acetyl-D-glucosamine)<sub>3</sub>, (GlcNAc)<sub>3</sub>] shown in green ( $\lambda_{\text{ex}}$  485 nm). Due to the lack of the target carbohydrate chitotriose in the skin tissue, no specific interaction between the lectin *Phytolacca americana* and the tissue is observed. The bright and stable fluorescence properties of the Atto 488 dye provide a strong fluorescent signal without requiring additional amplification steps. Further experiments with staining different fungal infected tissues were carried out. Similar results confirm this approach to be a successful and reliable way to detect fungi. This application may encourage scientists to investigate further histological phenomena by using lectin interactions.

The following Atto-dye lectin conjugates are now available from Sigma Life Science. Additional lectins and lectin conjugates available from Sigma Life Science may be found at [sigma.com/enzymeexplorer](http://sigma.com/enzymeexplorer)



**Figure 3:** Close-up of fluorescence image of human skin with fungal infection. It shows fine filaments of the fungi containing typical mycelium and individual fungi cells are clearly visible.

Description	$\lambda_{\text{ex}}/\lambda_{\text{em}}$ (nm)	Carbohydrate Specificity	Cat. No.	Package size
Lectin from <i>Artocarpus integrifolia</i> -Atto 594 conjugate	601/632 in PBS	O-Methyl- $\alpha$ -Galactose	76158	1 mg
Lectin from <i>Ulex europaeus</i> -Atto 488 conjugate	501/523 in PBS	$\alpha$ -L-Fucose	19337	0.5 mg
Lectin from <i>Ulex europaeus</i> -Atto 550 conjugate	554/576 in PBS	$\alpha$ -L-Fucose	94165	0.5 mg
Lectin from <i>Ulex europaeus</i> -Atto 594 conjugate	601/632 in PBS	$\alpha$ -L-Fucose	73873	0.5 mg
Lectin from <i>Phaseolus vulgaris</i> -Atto 488 conjugate (Leucoagglutinin)	501/523 in PBS	GlcNAc - Man	75319	1 mg
Lectin from <i>Phaseolus vulgaris</i> -Atto 550 conjugate (Leucoagglutinin)	554/576 in PBS	GlcNAc - Man	90852	1 mg
Lectin from <i>Phaseolus vulgaris</i> -Atto 647N conjugate (Leucoagglutinin)	644/669 in PBS	GlcNAc - Man	77363	1 mg
Lectin from <i>Phytolacca americana</i> -Atto 488 conjugate	501/523 in PBS	(GlcNAc) <sub>3</sub>	39905	1 mg
Lectin from <i>Phytolacca americana</i> -Atto 550 conjugate	554/576 in PBS	(GlcNAc) <sub>3</sub>	94816	1 mg
Lectin from <i>Phytolacca americana</i> -Atto 647N conjugate	644/669 in PBS	(GlcNAc) <sub>3</sub>	03065	1 mg
Lectin from <i>Triticum vulgare</i> -Atto 488 conjugate	501/523 in PBS	(GlcNAc) <sub>2</sub> , $\alpha$ -N-acetylneuraminic acid	16441	1 mg
Lectin from <i>Triticum vulgare</i> -Atto 532 conjugate	532/558 in PBS	(GlcNAc) <sub>2</sub> , $\alpha$ -N-acetylneuraminic acid	68917	1 mg

**Reference:**

Rhodes, J.M., Milton, J.D (1997): Lectin Methods and Protocols. Humana Press, Totowa, New Jersey.

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# ACCUSTAIN® Mucicarmine Stain

## Introduction

Sigma-Aldrich ACCUSTAIN® Mucicarmine Staining reagents are intended for staining tissue mucins of epithelial origin. Mucicarmine staining reagents are for "In-Vitro Diagnostic Use".

Tissue acid-mucopolysaccharides may be demonstrated using a variety of techniques. Mayer's mucicarmine procedure, as modified by Southgate, achieves excellent contrast between red staining mucin and other cellular components. Aluminium, contained in the formulation, is believed to form a chelate complex with mucin, to which carmine attaches by dye-lake formation. The stain displays a specificity towards mucins of epithelial origin, whereas mucins of fibroblastic origin stain poorly. Its major use may be in the identification of primary tumour sites and distinguishing mucin-negative undifferentiated squamous cell lesions from mucin-positive adenocarcinomas. The procedure described by Sigma-Aldrich is similar to that of Southgate. A stable solution of tartrazine has been substituted for metanil yellow. Included is a mucicarmine stain technique for rapid staining.

## Reagents

**Mucicarmine Stock Solution** Catalog No. HT3018 or HT30116. Carmine, certified, 10 g/L, aluminium hydroxide, 10 g/L, and aluminium chloride, 5 g/L, in ethanol, 50 % v/v.

**Weigert's Iron Hematoxylin Set** Catalog No. HT1079. (Part A & Part B).

**Tartrazine Solution** Catalog No. HT3024 or HT3028. Tartrazine (0.25 %), and acetic acid (0.25 %)

## Reagent Preparation

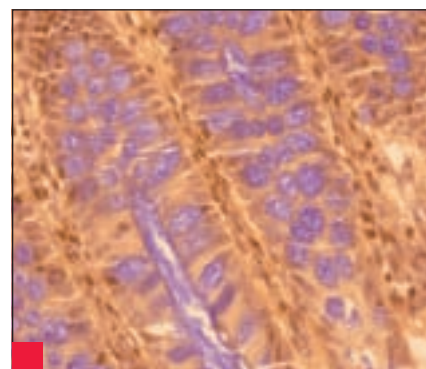
Prepare working mucicarmine solution by diluting mucicarmine stock solution 1:4 with tap water (e.g. 10 mL mucicarmine stock solution plus 30 mL tap water). Working solution is stable for 2–3 days when stored in the refrigerator. Prepare working Weigert's iron hematoxylin solution by adding equal amounts of Part A and Part B.

## ACCUSTAIN® Mucicarmine Stain

### Procedure

1. Deparaffinise tissue sections and hydrate with deionised water.
2. Stain in working Weigert's iron hematoxylin solution for 5 minutes.
3. Wash in running tap water for 5 minutes.
4. Stain in working mucicarmine solution for 30 minutes or longer at room temperature.
5. Rinse in deionised water.
6. Stain in tartrazine solution for 1–5 seconds.
7. Rinse slides, dehydrated through alcohol, clear in xylene and mount.

### Results



<b>Nuclei</b>	<b>Blue</b>
<b>Mucin</b>	<b>Deep rose to red</b>
<b>Capsule of Cryptococci</b>	<b>Deep rose to red</b>
<b>Other tissue elements</b>	<b>Yellow</b>

<b>Description</b>	<b>Cat. No.</b>
Mucicarmine Stock Solution	HT3018 (250ML)
	HT30116 (500ML)

# ACCUSTAIN® Reticulum Stain

## Introduction

Sigma-Aldrich Reticulum Stain is intended to demonstrate reticular fibres. Reticulum stain reagents are for "In Vitro Diagnostic Use".

The main function of reticular fibres is to provide support. They are normally found throughout the body, particularly in the liver, lymph nodes, spleen and kidneys. Ammoniacal silver stains are the most commonly used methods for demonstrating reticular fibres. In the procedure of Gordon and Sweets, tissue sections are oxidised by potassium permanganate with oxalic acid removing the excess potassium permanganate. Ferric ammonium sulphate acts as the sensitiser. After the silver impregnation, formalin is used to reduce the silver to its visible metallic form. Gold chloride tones the sections and any unreduced silver is removed by sodium thiosulphate. A counterstain may be used, if desired.

## Reagents

**Sodium Hydroxide Solution** Catalog No. HT1021, 3 % aqueous solution.

**Potassium Permanganate Solution** Catalog No. HT1022, 1 % aqueous solution.

**Oxalic Acid Solution** Catalog No. HT1023, 1 % aqueous.

**Ferric Ammonium Sulfate Solution** Catalog No. HT1024, 2.5 % aqueous solution.

**Silver Nitrate Solution** Catalog No. HT1025, 10 % aqueous solution.

**Gold Chloride Solution** Catalog No. HT1026, 0.2 % aqueous solution.

**Sodium Thiosulfate Solution** Catalog No. HT1027, 5 % aqueous solution.

## Reagent Preparation

Ammoniacal Silver Nitrate Solution:

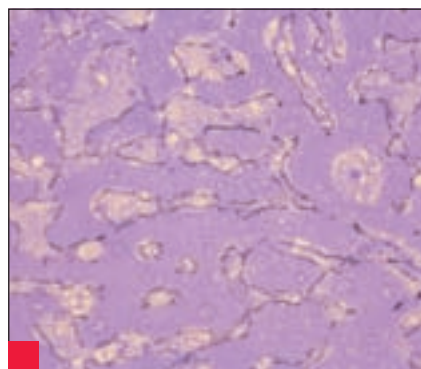
1. Pipet 5 mL silver nitrate solution in an Erlenmeyer flask.
2. While swirling flask, slowly add concentrated ammonium hydroxide, drop by drop, until precipitate formed is completely dissolved. Do not add excess.
3. Add 5 mL sodium hydroxide solution to flask. Black precipitate will form. Continuously swirl the flask and add concentrated ammonium hydroxide drop by drop until precipitate dissolves. Do not dissolve until completely clear. Faint cloudiness is desired.
4. Dilute to 50 mL with deionised water. Filter, use once and then discard.

## ACCUSTAIN® Reticulum Stain

### Procedure

1. Deparaffinise tissue sections and hydrate with deionised water.
2. Oxidise in potassium permanganate solution for 5 minutes.
3. Rinse in tap water for 2 minutes.
4. Bleach in oxalic acid solution for 2 minutes or until sections are colourless.
5. Wash in tap water for 2 minutes.
6. Sensitise in ferric ammonium sulphate solution for 15 minutes.
7. Rinse in several changes of deionised water.
8. Impregnate in ammoniacal silver nitrate solution for 2 minutes.
9. Rinse well in deionised water.
10. Reduce in 10 % formalin solution for 2 minutes.
11. Wash in tap water for 3 minutes.
12. Tone in gold chloride solution for 10 minutes.
13. Rinse in deionised water.
14. Place in sodium thiosulphate solution for 1 minute. Rinse in distilled water for 2 minutes.
15. Counterstain in a nuclear fast red solution for 3–5 minutes or an eosin Y solution for 1–2 minutes. Wash in water.
16. Dehydrate in alcohol, clear in xylene and mount.

### Results



**Reticulum  
Background**

**Black  
Pink to Rose  
(if counter stained with  
nuclear fast red)**

**Description**

**Cat. No.**

Reticulum Stain Kit

HT102A-1KT

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# Prestige Antibodies® as Organelle Markers for Sub-cellular Localisation Studies

## Introduction

Many characterisation studies of proteins require the localisation of specific cellular structures such as organelles. Co-localisation with organelle-specific antibodies corroborates sub-cellular location of the target protein and may thereby reveal the potential involvement of the protein in cellular processes.

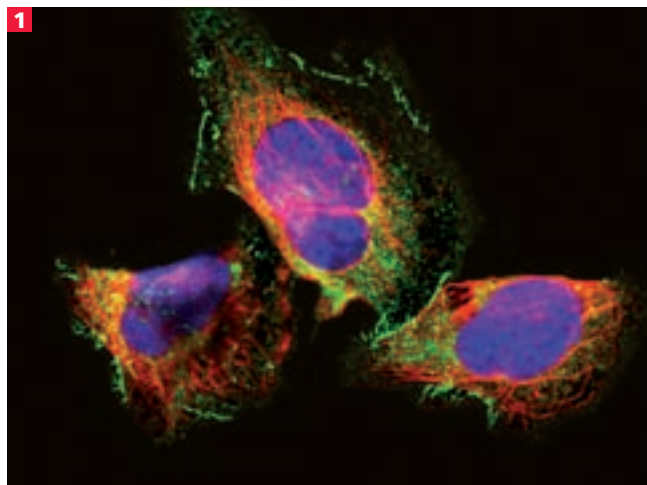
Amongst the most highly characterised antibodies in industry are the Prestige Antibodies®. Several Prestige Antibodies® have been shown to work as defined organelle markers. Each Prestige Antibody® has been uniquely tested and comes with over 700 images displaying expression and localisation of proteins in a large variety of normal human tissues, cancer cells and cell lines. All images are publicly available via the Human Protein Atlas ([www.proteinatlas.org](http://www.proteinatlas.org)), a web portal governed by the Human Protein Atlas (HPA) programme.

The HPA programme systematically explores the human proteome by combining highly characterised antibodies with massive protein profiling in human tissues and cells (1, 2). Although this multi-disciplinary research programme operates using a large-scale high-throughput approach, well-validated methods and applications such as immunohistochemistry (IHC) and immunofluorescence (IF) staining are used to ensure the high quality of these antibodies. The primary reagents generated from the HPA programme are affinity-purified polyclonal antibodies raised towards recombinant Protein Epitope Signature Tag (PrEST) antigens (3, 6).

All annotated images from the applications tested are made publicly available via the Human Protein Atlas ([www.proteinatlas.org](http://www.proteinatlas.org)), wherein more than two third of the characterised antibodies are Prestige Antibodies® (5).

## Immunofluorescence and Confocal Microscopy

In order to acquire information of organelle localisation and distribution into sub-cellular compartments, the performance of several anti-human protein-affinity reagents is tested in IF (4). Furthermore, the valuable features of confocal microscopy as an optical imaging technique are used to create high-resolution images of the many sub-structures in the cell. Combining the two offers a powerful tool for even deeper studies of the human proteome.



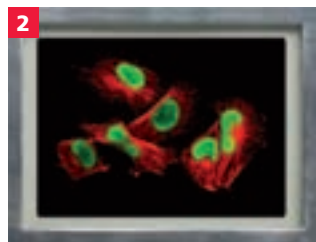
**Figure 1:** Staining of intercellular junctions in U-2 OS cells with organelle-specific Prestige Antibody anti-tight junction protein Zonula occludens protein 1. Cat. No. HPA001636.

The HPA programme has carefully selected three different human cell lines, A-431 epidermoid carcinoma, U-251 MG glioblastoma and U-205 osteosarcoma, for organelle mapping of the proteome (7). As Prestige Antibodies® are studied by IF staining, three well-characterised organelle markers for nuclei, microtubules and endoplasmic reticulum are used correspondingly as specific probes (8). The high-resolution confocal images are annotated based on sub-cellular localisation. In addition, staining intensity and characteristics are also part of an overall assessment as well as comparison to literature (if available). All these factors contribute to a final reliability score publicly available on the Web.

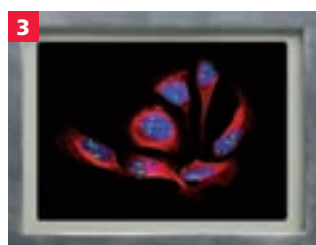
Among the Prestige Antibodies® tested in IF, a set of specific antibodies is classified as organelle markers. These probes have been subjected to the well-established quality control by the HPA programme, and are thus very well suited for sub-cellular localisation studies by IF.

# Prestige Antibodies® Organelle Markers

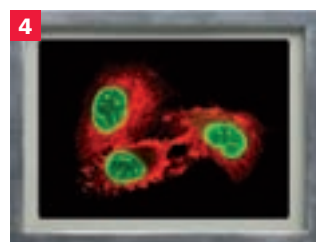
A selected set of organelle-specific Prestige Antibodies® is presented below.



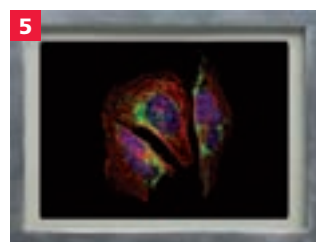
**Figure 2:** Nucleus: Anti-F-box only protein 18. Cat. No. HPA002844.



**Figure 3:** Nucleoli: Anti-visual system homeobox 2. Cat. No. HPA003436.



**Figure 4:** Nuclear Envelope: Anti-Sad1/unc-84-like protein 2. Cat. No. HPA001209.



**Figure 5:** Mitochondria: Anti-Stress-70 protein, Mitochondria precursor. Cat. No. HPA000898.

## Intercellular Junctions (Figure 1)

HPA001636, Anti-TJP1  
HPA001813, Anti-TJP2

## Nucleus (Figure 2)

HPA002844, Anti-FBXO18  
HPA000593, Anti-MECP2  
HPA001894, Anti-GPKOW  
HPA006801, Anti-XRCC4  
HPA006104, Anti-MYST4

## Nucleoli (Figure 3)

HPA007938, Anti-ZSCAN1  
HPA003436, Anti-CHX10

## Nucleus without Nucleoli

HPA004776, Anti-AKAP8  
HPA008784, Anti-FUS  
HPA006149, Anti-FUBP1

## Nuclear Envelope (Figure 4)

HPA008461, Anti-UNC84A  
HPA001209, Anti-UNC84B

## Golgi

HPA000992, Anti-GOLGA5  
HPA001677, Anti-GOLPH4

## Mitochondria (Figure 5)

HPA000898, Anti-HSPA9  
HPA006181, Anti-MRPL40  
HPA002932, Anti-TIMM9  
HPA000866, Anti-SYNJ2BP

## Peroxisomes

HPA007244, Anti-ACAA1

## Cytoplasm (Figure 6)

HPA001290, Anti-MTHFD1  
HPA001648, Anti-DDX3X  
HPA006731, Anti-USP10

## Focal Adhesions (Figure 7)

HPA004835, Anti-ZYX  
HPA005724, Anti-VASP  
HPA001349, Anti-MYH6

## Plasma Membrane

HPA001672, Anti-SLC9A3R2  
HPA003097, Anti-NF2

## Endoplasmic Reticulum (Figure 8)

HPA003230, Anti-PDIA3  
HPA003906, Anti-BCAP31  
HPA003178, Anti-KTN1

## Centrosomes (Figure 9)

HPA003647, Anti-DNAL4

## Cytoskeleton-Microtubules (Figure 10)

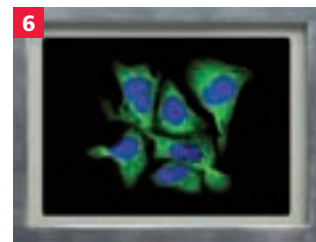
HPA006376, AntiTGFB111

## Cytoskeleton-Actine Filaments

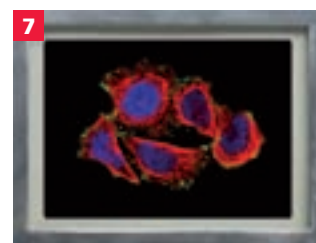
HPA002925, Anti-FLNA  
HPA006035, Anti-ACTN1

## Cytoskeleton-Intermediate Filaments

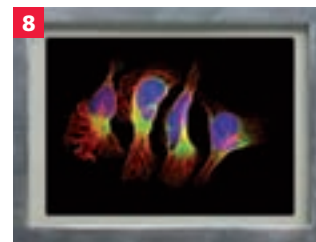
HPA001762, Anti-VIM  
HPA007007, Anti-NES



**Figure 6:** Cytoplasm: Anti-ATP-dependent RNA helicase DDX3X. Cat. No. HPA001648.



**Figure 7:** Focal Adhesions: Anti-Zyxin. Cat. No. HPA004835.



**Figure 8:** Endoplasmic Reticulum: Anti-Protein disulfide-isomerase A3 precursor. Cat. No. HPA003230.



**Figure 9:** Centrosomes: Anti-Dynein light chain 4, axonemal. Cat. No. HPA003647.

# Immunofluorescence

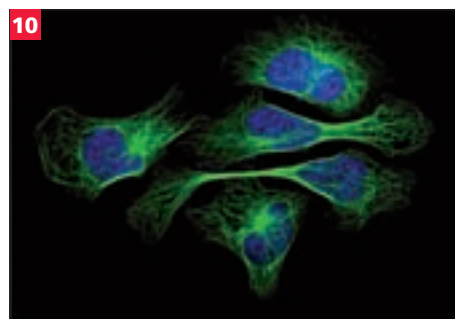
## Introduction

The choice of fixative and detergent for cell permeabilisation is a crucial factor in immunofluorescence labelling. Based on thorough studies by the HPA programme, the chosen standard procedure for the high throughput effort is Triton® permeabilization (4). Three cell lines are cultured in vitro, fixated, permeabilised and stained according to the standardised procedure hereby presented. In addition to staining with Prestige Antibodies®, cells are treated with two organelle probes specific for the endoplasmatic reticulum (ER) and microtubules, as well as counterstained with the nuclear probe DAPI.

## Procedure

1. Cells are seeded (10,000–15,000 cells per well) and incubated at 37 °C in humidified air with 5.2 % CO<sub>2</sub>, for 4 hours.
2. The cells are washed in PBS.
3. The cells are fixed for 15 minutes in ice cold 4 % paraformaldehyde (pH 7.2–7.3) in growth medium supplemented with 10 % fetal bovine serum (FBS).
4. The cells are permeabilised 3x5 minutes with 0.1 % Triton X-100 in PBS.
5. The cells are washed once with PBS.
6. Primary antibodies are diluted to 2 ug/ml in blocking buffer (PBS supplemented with 4 % FBS) and incubated overnight at 4 °C.
7. The following day, the cells are washed 4x 10 minutes with PBS.
8. Secondary antibodies are diluted to 1 ug/ml in blocking buffer and incubated for 1.5 hours in room temperature.
9. The cells are counterstained for 4 minutes with the nuclear marker DAPI (0.3 µM in PBS).
10. The cells are washed 4x 10 minutes with PBS before mounted.

## Results



**Figure 10:** Immunofluorescent staining of human cell line U-2 OS with specific organelle marker shows positivity in cytoskeleton (microtubule, green).

### Description

Prestige Antibody  
Anti-Androgen receptor co-activator ARA55 isoform 2

### Cat. No.

HPA006376

## References

- 1] Kampf, C. *et al* (2004): Antibody-based tissue profiling as a tool in clinical proteomics. *Clin Proteomics* 1:285–299
- 2] Uhlén, M. *et al* (2005): A human protein atlas for normal and cancer tissues based on antibody proteomics. *Mol Cell Proteomics* 4(12):1920–32
- 3] Hober, S. and Uhlén, M. (2007): Human protein atlas and the use of microarray technologies. *Curr Opin Biotech* 19:1–6
- 4] Barbe, L. *et al* (March 2008): Towards a confocal sub-cellular atlas of the human proteome. *Mol Cell Proteomics* 7(3):499–508
- 5] Berglund, L. *et al* (2008): A gene-centric protein atlas for expression profiles based on antibodies. *Mol Cell Proteomics* 7:2019–202
- 6] Pontén, F. *et al* (2008): The Human Protein Atlas – a tool for pathology. *J Pathol* 216: 387–393
- 7] Strömberg, S. *et al* (2009): Selective expression of Syntaxin-7 protein in benign melanocytes and malignant melanoma. *J. Proteome Res.* 8 (4), pp 1639–1646
- 8] Stadler, C. *et al*: Fixation protocols for systematic localisation studies using immunofluorescence. Submitted.

# Development of a Fast and Simple One-Dimensional Separation Approach for the Detection of Low Abundance Plasma Proteins

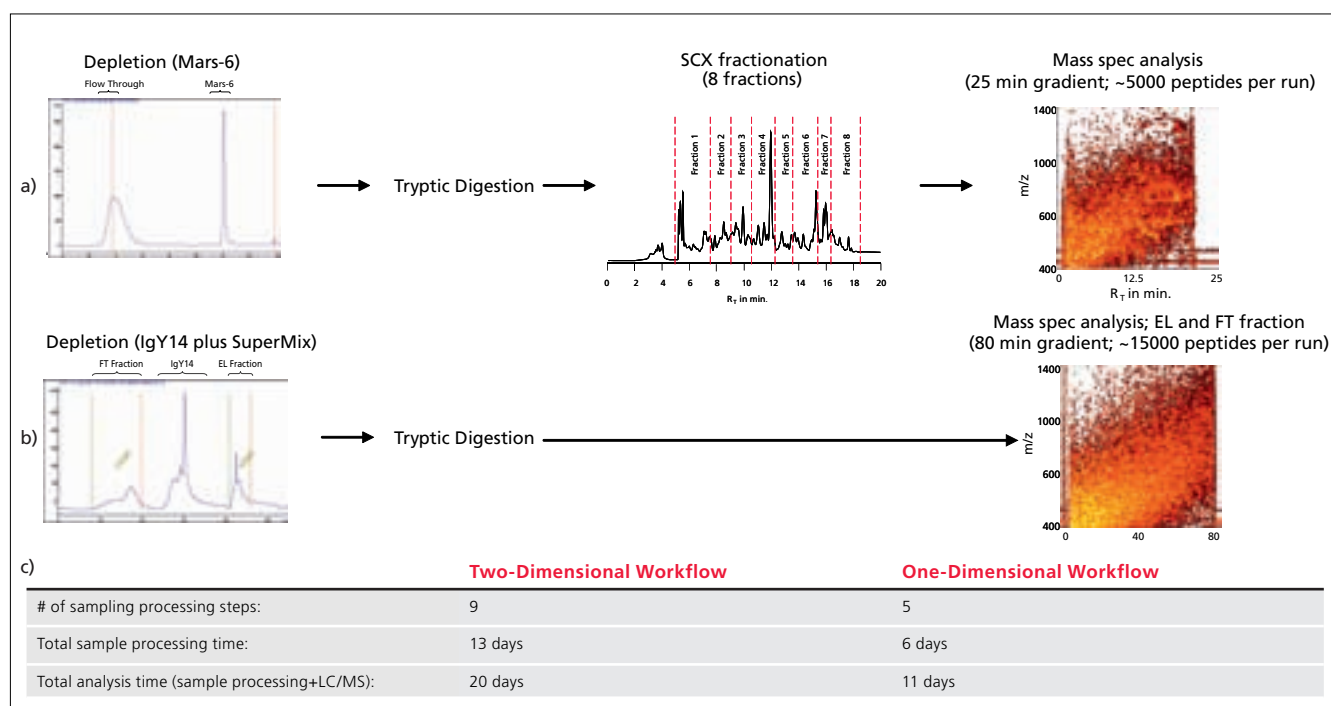
Michael Schirm, Dmitri Sitnikov, Enrique Escobar, Tam Lehuu, Chunyan Li and Joanna Hunter  
Caprion Proteomics, Inc. 7150, rue Alexander Fleming, Montreal, QC, Canada

## Introduction

Due to the complexity of the plasma proteome, it represents one of the most challenging proteomes to analyse. In order to detect low abundance proteins, time-consuming two-dimensional separation techniques such as antibody-based depletion methods in combination with SCX chromatography are often used prior to LC/MS analysis. In this study, a faster and simpler one-dimensional separation workflow using the Genway IgY-14 plus SuperMix depletion columns were developed. These methods were evaluated by a protein spiking experiment and an Alzheimer's disease biomarker study. Each method was tested for the detection of low abundance plasma proteins as well as additional analytical criteria such as number, reproducibility and intensity CV of detected peptides, and total processing and analysis time.

## Methods

A pool of human plasma was spiked with five non-human proteins (ovotransferrin, alcohol dehydrogenase, phosphorylase B, beta-galactosidase and hexokinase) at eight concentrations ranging from 2 pg/ $\mu$ L to 100 ng/ $\mu$ L. Samples were processed by a one-dimensional (1-D) and a two-dimensional (2-D) separation workflow (**Figure 1**). For the two-dimensional separation workflow, samples were depleted in triplicate using the Agilent MARS-6 depletion column. Depleted samples were digested with trypsin, desalted and fractionated by strong cation exchange (SCX) chromatography into eight fractions prior to LC-MS and MS/MS analysis (25 min gradient). For the one-dimensional separation workflow, samples were depleted using the Genway IgY-14 plus SuperMix depletion columns. The flow-through (FT) fraction (proteins binding neither to the IgY-14 and SuperMix depletion column) and eluate (EL) fraction (proteins binding to the SuperMix depletion column) were collected, digested with trypsin, desalted and analysed by LC-MS and MS/MS (80 min gradient).



**Figure 1:** Overview of the one-dimensional and two-dimensional separation workflows. The 2-D separation includes fractionation of peptides by SCX followed by a short LC-MS analysis, while the 1-D separation eliminates the SCX fractionation step, but increases the length of the LC-MS analysis. The time saving of the 1-D separation workflow is shown in part (c) ( $n = 60$  samples). (a) Two-Dimensional Workflow; (b) One-Dimensional Workflow.

For the biomarker study, plasma was analysed from four groups of patients:

- 1) Alzheimer's disease untreated
- 2) Alzheimer's disease treated (donepezil)
- 3) Mild cognitive impairment
- 4) Healthy individuals

There were ten samples in each condition. Samples were processed with either the one- or two-dimensional separation workflow as described above.

All data was analysed using in-house developed software as well as with commercial statistical analysis packages.

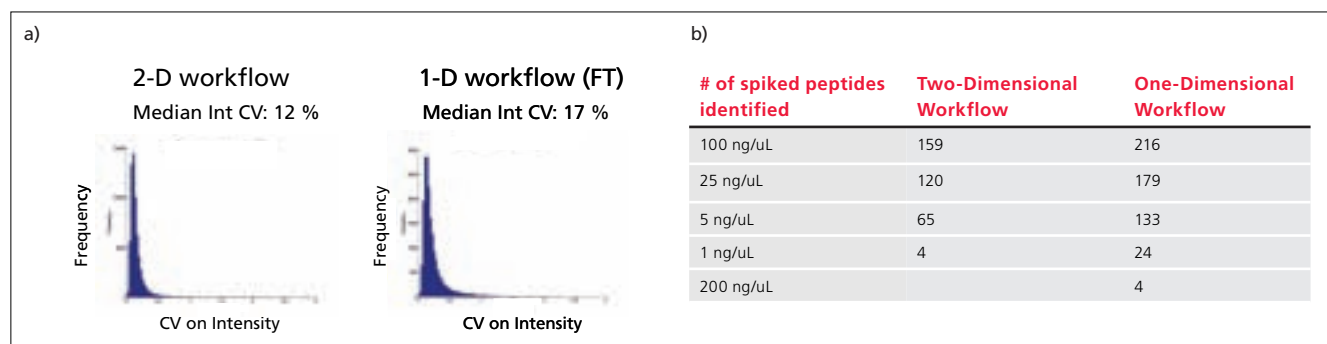
## Results

**Iterative exclusion MS/MS:** Exclusion MS/MS runs of a pooled plasma sample identified 304 unique proteins for samples processed with the 2-D workflow and 542 unique proteins for samples processed with the 1-D workflow. After the 1-D separation, 472 proteins were identified in the flow-through fraction and 169 proteins in the eluate fraction, while 99 proteins were identified in both fractions (FT and EL).

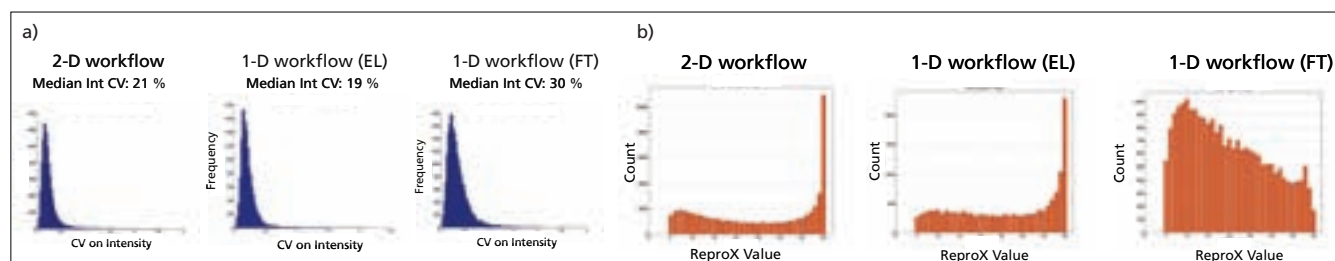
**Spiking experiment:** The intensity variability of detected peptides was slightly higher for the one-dimensional separation approach (17 %) compared to the two-dimensional separation approach (12 %) (Figure 2a). However, the one-dimensional separation workflow allowed the detection of more spiked peptides and approximately five times lower abundance proteins.

**Alzheimer's Disease Biomarker Study:** For the AD biomarker study, increased intensity CVs were observed for the FT fraction of the 1-D separation workflow as compared to the EL fraction of the 2-D separation workflow (Figure 3a). In addition, the FT fraction showed a set of peptides, which were non-reproducible for the analysed samples (Figure 3b). Expression analysis of the reproducible peptides identified 814 differentially expressed peptides for the 2-D separation workflow. For the 1-D separation workflow, 370 differentially expressed peptides were identified in the eluate fraction and 1222 peptides in the flow-through fraction (Figure 4a). Generation of multi-dimensional scaling (MDS) plots of the differentially expressed peptides allowed the separation of the different patients groups with both workflows. MDS analysis of the FT fraction of the 1-D separation workflow is shown in Figure 4b.

Differentially expressed peptides were targeted for sequencing by tandem mass spectrometry for the identification of over-expressed proteins. Several proteins were identified with known Alzheimer's disease link such as properdin, mannose binding protein, calmodulin, carbonic anhydrase, clusterin, serotransferrin and transthyretin.

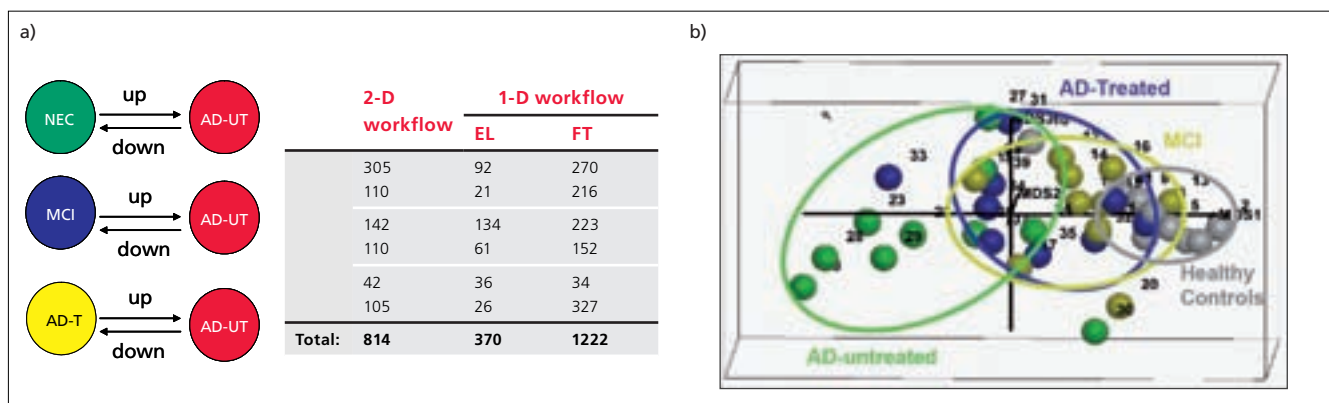


**Figure 2:** Peptide intensity variability and detection limits for the spiking experiment. The intensity distribution and median CV of detected study peptides (SP) is shown in (a). The number of spiked peptides identified for each workflow is shown in (b).



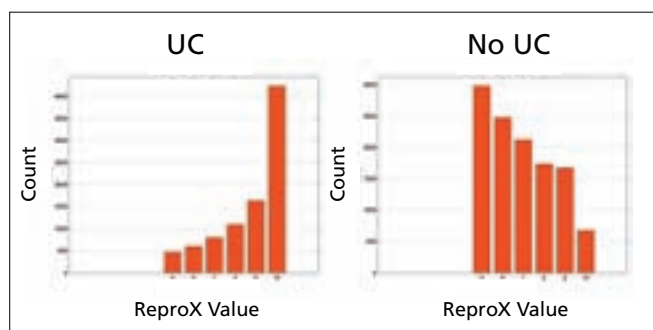
**Figure 3:** Peptide intensity variability and peptide reproducibility for the AD biomarker study:

- a) Intensity distribution and median CV of detected peptides for the 2-D and 1-D workflows.
- b) Study peptide reproducibility for the 2-D and 1-D workflows.



**Figure 4:** Differentially expressed peptides and MDS plots for the AD biomarker study: a) Number of differentially expressed peptides identified between the four groups of patients ( $dI \geq 1.5$ , p-value 0.05, overexpressed  $\geq 7$  out of 10 patients); b) MDS plot of differentially expressed peptides for the flow-through fraction of the 1-D workflow.

The non-reproducible peptides observed for the FT fraction was hypothesised to come from low molecular weight compounds endogenous in plasma. To test this, 10 samples from the AD study were reprocessed, and low molecular weight compounds were removed by ultra-centrifugation (5 kDa cut off) prior to digestion. Results of this experiment are shown in **Figure 5**. Median intensity CV decreased from 30 % to 22 %, and an improved peptide reproducibility was observed for the ultracentrifuged samples.



**Figure 5:** Peptide reproducibility with and without ultracentrifugation (UC). The study peptide reproducibility of 10 AD plasma samples reprocessed with and without ultracentrifugation is shown (1-D workflow; FT fraction).

### Conclusion

The one-dimensional separation approach offers several advantages as compared to the two-dimensional separation workflow:

- It allows the detection of lower abundant plasma proteins
- The number of sample processing steps and sample processing time is reduced by about half
- A greater number of proteins are identified

Removal of the low molecular weight compounds prior to digestion appears to eliminate most of the non-reproducible peptides observed for the AD biomarker study.

### Acknowledgments

The authors would like to thank Dr. Sergey Sikora (Genway Biotech, Inc.) for providing the IgY-14 and SuperMix depletion columns.

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## Detecting Disease-Carrying Ticks using a New Microbiological Rapid Test System

Disease-carrying ticks are becoming more common as global climate change alters our weather. Therefore, there is greater concern about the increased potential for parasitic diseases infecting humans after bites from these organisms. But today, detection systems are available for the rapid identification of disease-causing microorganisms found in ticks.

By Manuela Fabienke, Scanbec m.fabienke@scanbec.de

Scanbec GmbH has developed and produced rapid innovative molecular test systems for the detection of microorganisms. In 2003, they developed a test system called HybriScan®, which is now distributed by Sigma-Aldrich. There are applications for the HybriScan® system in the food and beverage industry, water analysis and medical diagnostics. The adaptability of HybriScan® technology makes possible the development of beneficial universal methods and is a good base for developing new testing systems. Scanbec, an accredited laboratory, also provides micro- and molecular-biological services.

Due to increasing temperatures and changing climatic conditions, ticks have become more and more dangerous. These organisms are able to transmit parasitic diseases, the most prominent of which is called borreliosis or Lyme disease.

Removing and then analysing the tick itself, rather than testing the human who has been bitten, for evidence of pathogens is of extraordinary value to both the attending doctor and the concerned person. Everyone receives fast and reliable results, allaying fears about infection faster than ever before. Scanbec uses a new rapid test system called a rapid stripe assay that is able to detect and identify borreliosis (Lyme disease) pathogens in ticks within two hours; the test is conducted in collaboration with the company Analytik Jena AG.

There has been a proven increase in tick populations within the last few years; thus the risk of being bitten by a tick has drastically increased as well. Before the new system, people who were bitten by ticks had to wait four to six weeks after being bitten to be tested. The waiting time served to allow the person's immune system time to produce antibodies against the pathogens; antibodies that would then be detected using common blood tests. The direct detection of pathogens in the tick enables diagnosis and treatment of infections in its earliest stages.



**Figure 1:** *Borrelia* lateral flow assay.

Using this new test method, the removed tick can be fixed on a strip of tape and sent to Scanbec's laboratory by regular mail.

The detection of *Borrelia* transmitted via ticks occurs by a direct amplification of the target DNA isolated from the tick sample. The analysis is performed in three steps. First, the tick is mechanically destroyed in order to isolate total DNA from the tick tissue. Then, the *Borrelia* DNA is specifically amplified using PCR. During PCR, *Borrelia*-specific primers are used to amplify the pathogen's DNA, detecting and amplifying all different *Borrelia* species. The last step entails the detection of the amplified *Borrelia* DNA using a stripe assay (lateral flow assay). The amplified *Borrelia* DNA is applied to the test strip and the results become visible after a few minutes. Two violet lines indicate the presence of *Borrelia* species bacteria in the tick. One violet line indicates that the tick was free of all *Borrelia* species (**Figure 1**).

Tick species	Number		
	Total	<i>Borrelia</i> positive	<i>Borrelia</i> species (determined via sequencing)
<i>Dermacentor reticulatus</i>	36	2	
<i>Ixodes hexagonus</i>	1	-	
<i>Haemaphysalis concinna</i>	5	1	n.n.
<i>Ixodes ricinus</i>	358	56	<i>B. afzelii</i> , <i>B. garinii</i>
<b>Total</b>	<b>400</b>	<b>59</b>	

**Table 1:** Results of the validation study performed by Analytik Jena AG.

The accuracy of the rapid tick test for the detection and identification of *Borrelia* species was validated by the company Analytik Jena AG. Four hundred ticks were tested. All positive results were then sequenced to verify the obtained results and to classify the detected *Borrelia* species. The sample pool of 400 included ticks of the following types: *Ixodes ricinus*, *Dermacentor reticulatus*, *Ixodes hexagonus* and *Haemaphysalis concinna*.

After the detection of *Borrelia* in a tick, the identification stage of the test system begins, and the particular species of *Borrelia* is determined by sequencing. With these results, the attending physician can predict which symptoms are likely to manifest in the patient. For example, *B. garinii* mediates neuroborreliosis (a disorder of the central nervous system) and *B. afzelii* is known to mediate erythema migrans (which causes patches of reddened skin 5 to 6.8 cm in diameter). Since treating borreliosis is both complicated and difficult, knowing of which *Borrelia* species has infected the patient can be of great help.

It is important to note that a positive result for the presence and identification of *Borrelia* species is only the first step in the larger process of handling the infection. Scanbec recommends

in all cases of positive *Borrelia* test results, that the person in question follow up promptly with a doctor for examination and further testing, including early-stage blood or serum sample collection to monitor the patient's serological processes (*Borrelia* antibody status), to see whether symptoms of borreliosis emerge and to ensure effective treatment with the appropriate antibiotics.

Scanbec also offers test systems for the detection and identification of pathogenic *Rickettsia*, *Babesia* and *Anaplasma* species in ticks. These tests work on the same principle as the *Borrelia* test system, differing only in the species-specific PCR primers used for amplification of the DNA of the respective microorganisms.

Like *Borrelia*, all three of these pathogens can be transmitted by ticks to humans. A growing tick population, caused by global climate change, increases the likelihood of tick-borne infections like borreliosis/Lyme disease, tick-borne meningoencephalitis and rickettsiosis in humans.



*Rickettsia* species are found in the intestine and intestinal epithelia of arthropods like ticks. With this disease, detection during the early stages of infection means an easy treatment with antibiotics, thus the new rapid test time is key to a better outcome for the patient. A recent survey on the proliferation of pathogens in ticks revealed that *Rickettsia* species are occurring with unexpected frequency in ticks. *Rickettsia* are able to survive only in endothelial cells; therefore, traditional testing for these bacteria involved proliferation by means of cell culture, which is highly time- and cost-intensive. Further, reliable confirmation of the presence of *Rickettsia* is only possible by nucleic-acid-based detection by PCR. This new system provides a faster and more cost-effective alternative to these traditional tests.

The new tick test for the detection of *Rickettsia* was also validated by Analytik Jena AG. Again, a sample pool of 400 ticks was examined for the occurrence of *Rickettsia* to evaluate the specificity and accuracy of *Rickettsia* detection results.

*Rickettsia* positive samples were sequenced to verify the obtained results and to classify the detected *Rickettsia* species. The following tick species were examined: *Ixodes ricinus*, *Dermacentor reticulatus*, *Ixodes hexagonus* and *Haemaphysalis concinna*.

Ticks are also able to transmit animal pathogens like *Babesia bovis* and *Babesia bigemina* (tick fever or cattle fever). Both *Babesia* species are animal pathogens that usually affect mice, cattle, horses, and dogs. In exceptional cases, however, humans can be infected after being bitten by a tick carrying the protozoa. *Babesia divergens* and *Babesia microti* are also widespread through Europe and North America and can cause influenza-like symptoms such as fever, ague, arthralgia, thrombopenia, haemolytic anaemia and haemoglobinuria in humans.

Ticks feeding on infected animals absorb the *Babesia* with erythrocytes. Within the intestine of the tick, the *Babesia* bacteria are released from the erythrocytes and start to proliferate. The *Babesia* then divide into thousands of sporozoites, the infectious stage of the parasite, in the saliva of the tick and are transmitted to a new host when the tick moves on.

*Anaplasma phagozytophila* is able to proliferate in leukocytes, especially in granulocytes. Transmission of anaplasmosis occurs, according to the pertinent literature, mainly via ticks. *Anaplasma phagocytophila* (formally known as *Ehrlichia phagocytophila*) causes human granulocytic anaplasmosis (HGA) and is prominent in southern and eastern Europe. Humans infected with anaplasmosis exhibit symptoms like fever, head and limb pain, gastrointestinal and pulmonary symptoms, and exanthema.

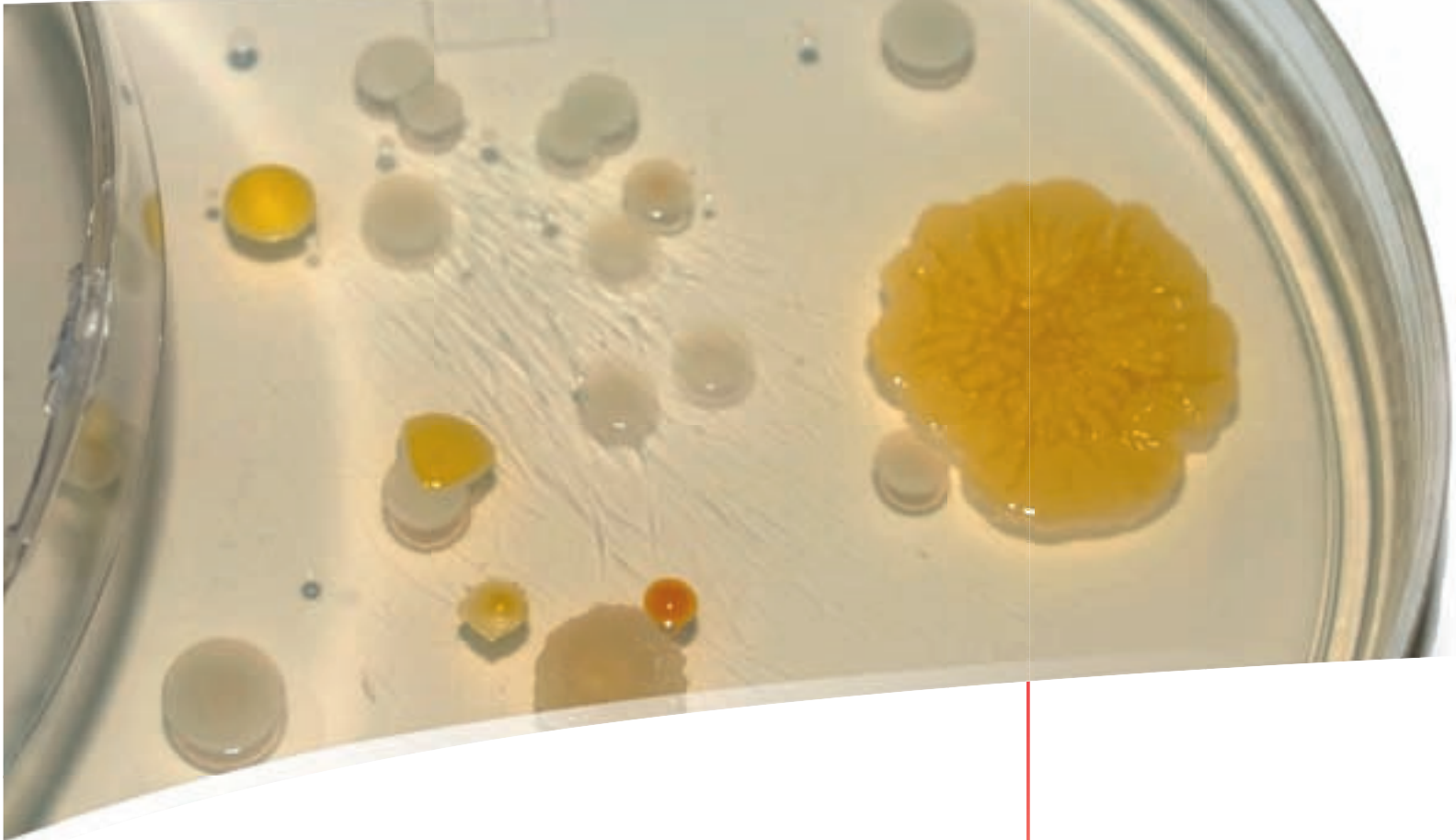


Tick species	Number		
	Total	<i>Rickettsia</i> positive	<i>Rickettsia</i> species (determined via sequencing)
<i>Dermacentor reticulatus</i>	36	16	<i>Rickettsia slovacica</i> , <i>Rickettsia sibirica sibirica</i> (Subtype Chabarowsk)
<i>Ixodes hexagonus</i>	1	-	
<i>Haemaphysalis concinna</i>	5	-	
<i>Ixodes ricinus</i>	358	39	<i>Rickettsia helvetica</i>
<b>Total</b>	<b>400</b>	<b>55</b>	

**Table 2:** Results of the validation study performed by Analytik Jena AG.

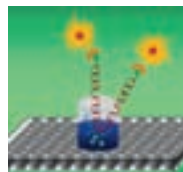
# HybriScan® – Rapid Test Systems

for the Detection and Identification of Microorganisms

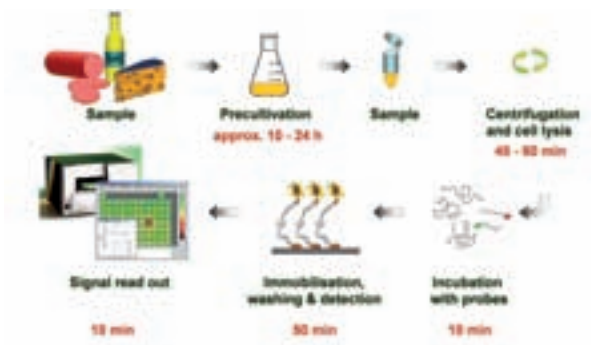


## The technology

HybriScan® technology is based on the detection of target molecules, specific for the microorganism of interest, with capture and detection probes by means of a so-called sandwich hybridisation. The signal read-out is triggered optically by an enzymatically generated colour change.



## Easy sample preparation:



- Rapid, sensitive and reliable, without PCR
- Saves up to 10 days in comparison to cultivation-based assays
- Live/dead discrimination
- Easy handling with standard lab equipment, like 96-well-plate reader
- Quantifiable by standards

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