

# Next Generation Multiparameter IFA Technology



*Simply innovative diagnostics*

**BytoBead<sup>®</sup>**  
for manual and automated assessment

Patents: EP 2362222, EP 3460750 (pending)



# CytoBead<sup>®</sup> Technology

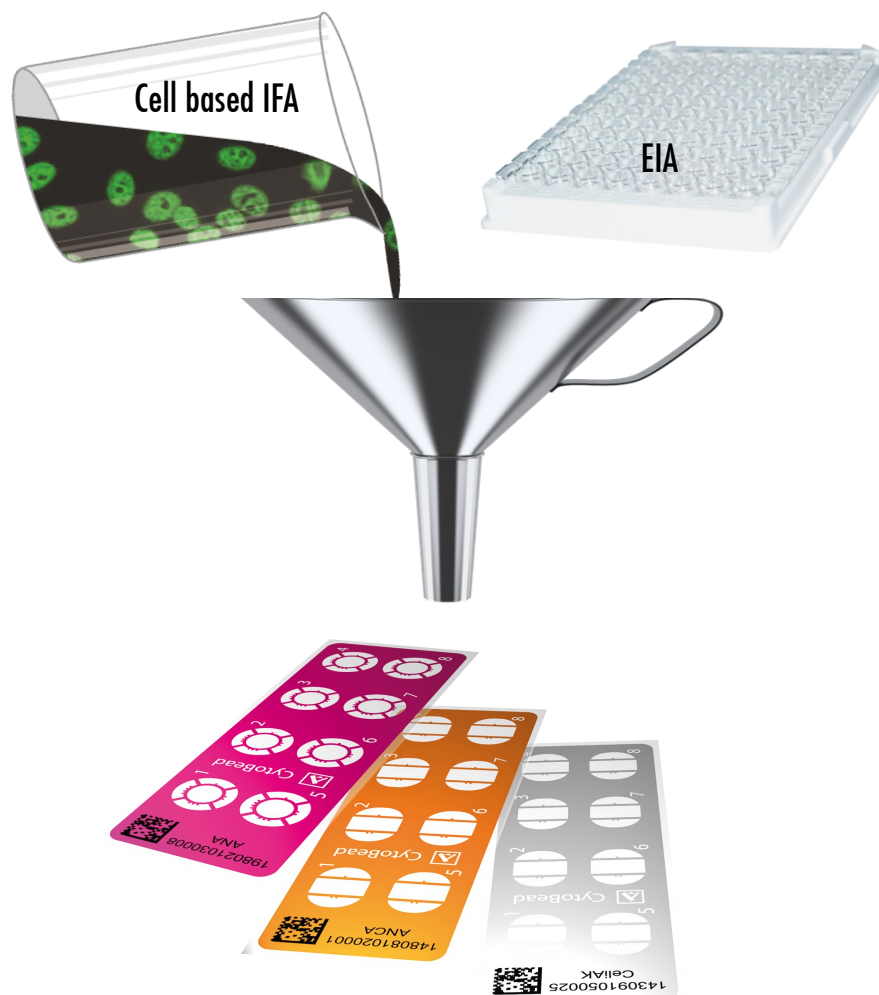
## Next Generation IFA

Combination of screening and confirmation in one step.

Automated evaluation **using** AKLIDES<sup>®</sup> **or** akiron<sup>®</sup> systems

Screening and  
Confirmation:  
One Assay

Screening + Confirmation



**CytoBead<sup>®</sup>**

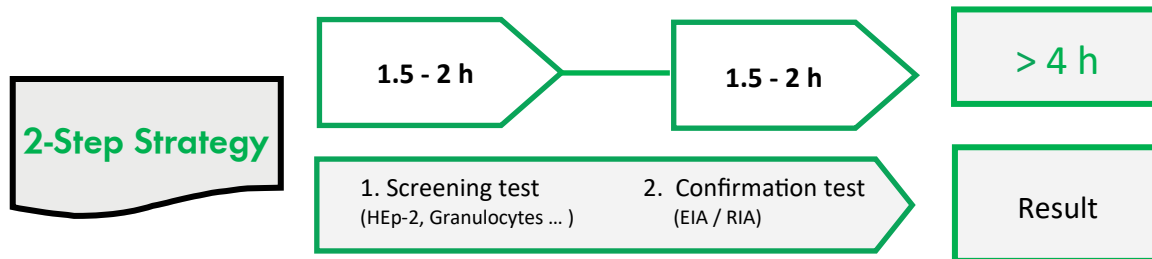
Available for:

ANA | ANA 2 | ANA DFS-70 | ANCA | CeliAK | RPGN

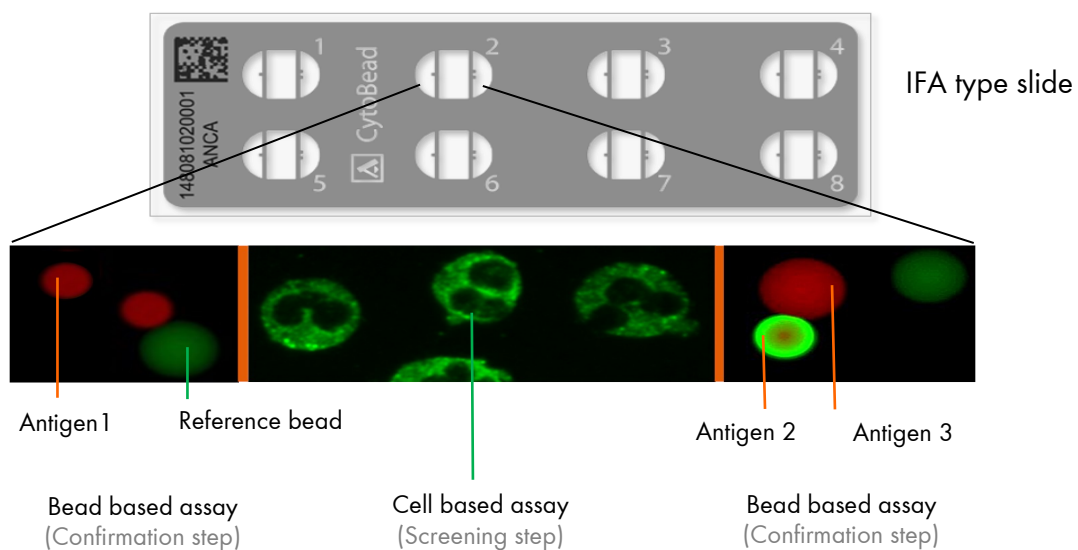
# CytoBead<sup>®</sup> Technology



## Standard workflow in Autoimmune Diagnostics



## How do CytoBead<sup>®</sup> assays work?



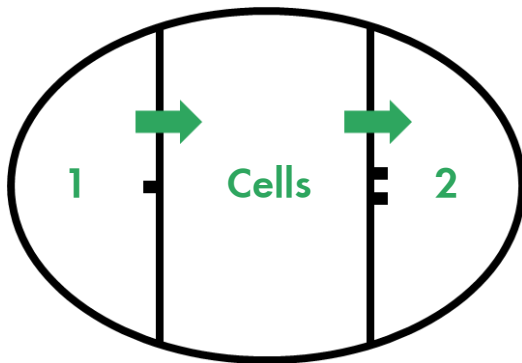
### Compartmented slide well:

- Screening on cells (classic IFA) in one compartment
- Confirmation with a bead based assay in the others

# Manual CytoBead<sup>®</sup> Evaluation

## Bead compartment guides for better orientation

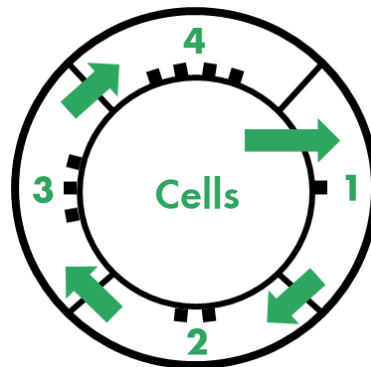
### Examples



Reading direction →

Compartment 1 to cells to compartment 2

■ Compartment marker

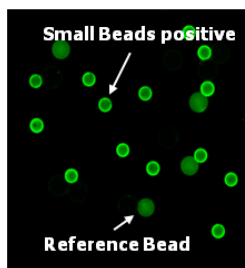


Reading direction →

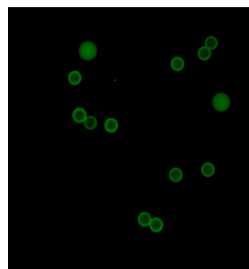
Cells to compartment 1 to 2 to 3 to 4

■ Compartment marker

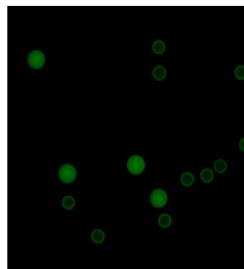
## Semi-quantitative evaluation of fluorescence intensity



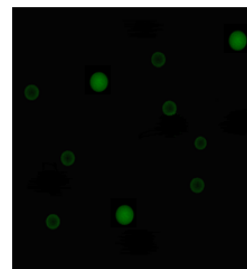
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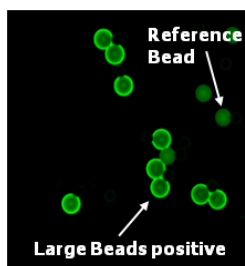


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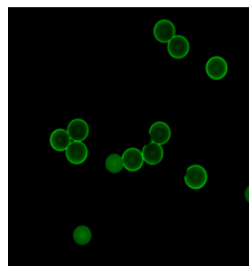


+/-

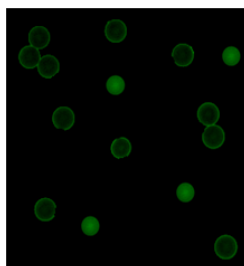
small beads  
(= smaller than  
reference beads)



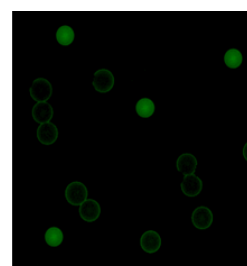
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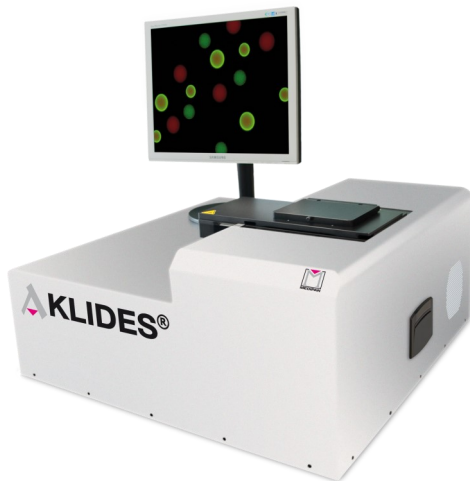
+/-

large beads  
(= larger than  
reference beads)

Enlarged images (10x objective, obtained with AKLIDES<sup>®</sup>, MEDIPAN GmbH)

# Automatic CytoBead<sup>®</sup> Evaluation

## Evaluation with the Technology Platforms AKLIDES<sup>®</sup> and *akiron*<sup>®</sup> NEO

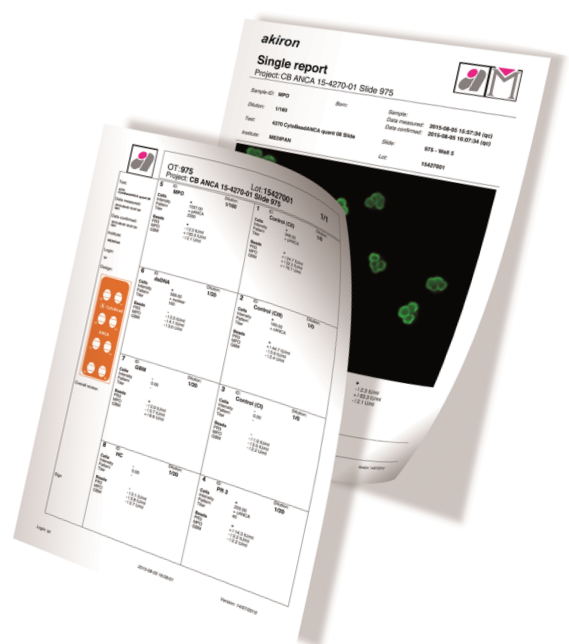


Patents: EP 2362222, EP 3460750 (pending)

## Technical features

- Fully automated screening and objective evaluation of cells and tissues.
- Automated bead identification and measurement of ligand fluorescence intensity.
- Standardized and reliable results between different instruments due to intensity calibration.
- ELISA typical quantification of antibodies in U/ml or IU/ml with lot-specific standard curves using 4 point calibration (selected tests).
- Automatic titer determination → only one sample dilution is required.
- Overall report of all relevant quantitative results in pdf- and xls-formats.
- Single-well report in pdf-format (including cell images).

## Single and overall report



# CytoBead® ANA / ANA 2

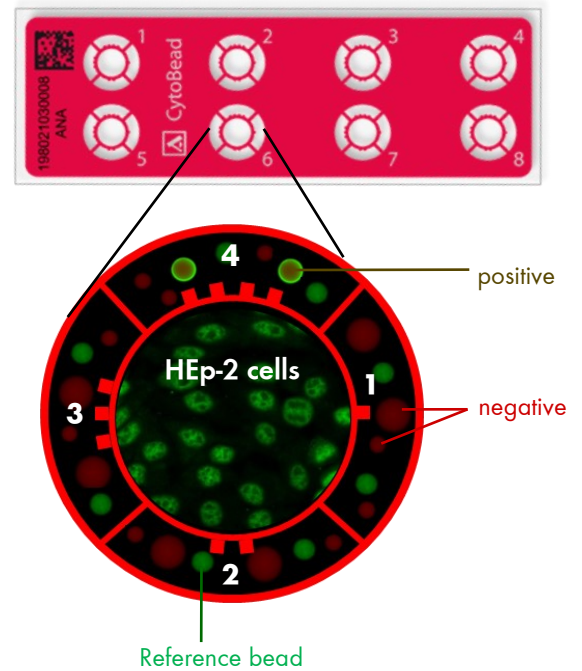
## Fast and easy ANA diagnostics

ANA (Anti-Nuclear Antibodies) are autoantibodies (AAb) which recognize conserved nuclear antigens. ANA show a characteristic staining of nuclear structures with indirect immunofluorescence on human epithelial cells (HEp-2). The confirmation of ANA is done in accordance to the target antigens.

Unique combination of HEp-2 cells with antigen-coated microbeads

### Advantages of CytoBead® ANA / ANA 2

- **Screening** with standardized HEp-2 cells
- **Confirmation** of 8 ANA-specific antigens



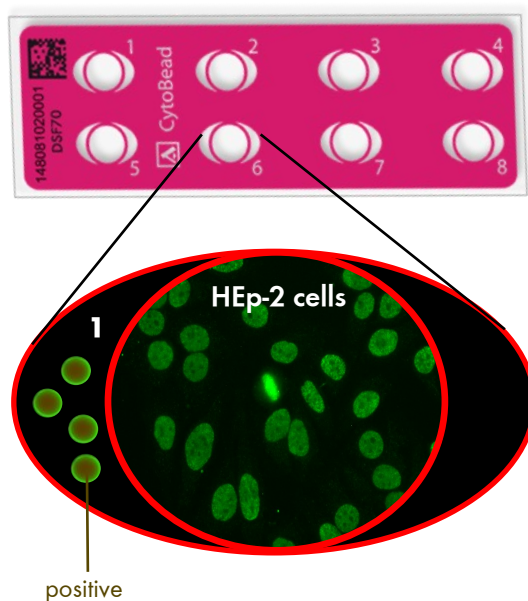
## CytoBead® ANA / ANA 2

HEp-2 cell pattern	Compartment	Bead (positive)	Quantification	Antigen	Clinical relevance
Homogeneous	3		IU/ml	dsDNA	Systemic lupus erythematosus (SLE)
			U/ml	Scl-70	Marker for progressive systemic sclerosis (PSS)
Speckled	2		U/ml	Sm	Sm antibodies highly specific for SLE; high anti-nRNP titers specific for mixed connective tissue disease (MCTD) together with other ANAs in rheumatoid arthritis (RA), SLE, PSS
			U/ml	nRNP	
	4		U/ml	Ro60/SS-A	Often in primary Sjögren's syndrome, anti-SS-A often in neonatal lupus
			U/ml	Ro52/SS-A	
	1		U/ml	La/SS-B	
Centromere	1		U/ml	CENP-B	Marker for CREST syndrome, rarely in diffuse scleroderma and Raynaud's phenomenon

## CytoBead® ANA 2, Jo-1 microbead replaces CENP-B microbead

Cytoplasmic	1		U/ml	Jo-1	Polymyositis, dermatomyositis
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# CytoBead<sup>®</sup> ANA DFS-70



## Exclusion of „false positive“ ANA results


Up to 20 % of positive patterns in indirect immunofluorescence on HEp-2 cells may be caused by antibodies to DFS-70, confusing the assignment of positive results to systemic rheumatic diseases. These antibodies show dense fine speckled nuclear pattern combined with speckled mitotic chromatin zone. Fluorescence staining also on the added DFS-70 beads allows the exclusion of these results from rheumatologic screening.

Unique combination of HEp-2 cells with DFS-70-coated microbeads

## Advantages of CytoBead<sup>®</sup> ANA DFS-70

- **ANA Screening** with standardized HEp-2 cells
- **Exclusion of non-related patterns** using the DFS-70 beads

## CytoBead<sup>®</sup> ANA DFS-70

HEp-2 cell pattern	Com-part-ment	Bead (positive)	Quantification	Antigen	Clinical relevance
<b>Dense fine speckled</b>	<b>1</b>		Not available yet	DFS-70	Very rare in systemic rheumatic diseases, found in different autoimmune diseases and malignancies, also present in healthy people
<b>Other patterns</b>	See CytoBead <sup>®</sup> ANA and ANA 2				

# CytoBead<sup>®</sup> ANCA / RPGN

## Fast and easy ANCA diagnostics

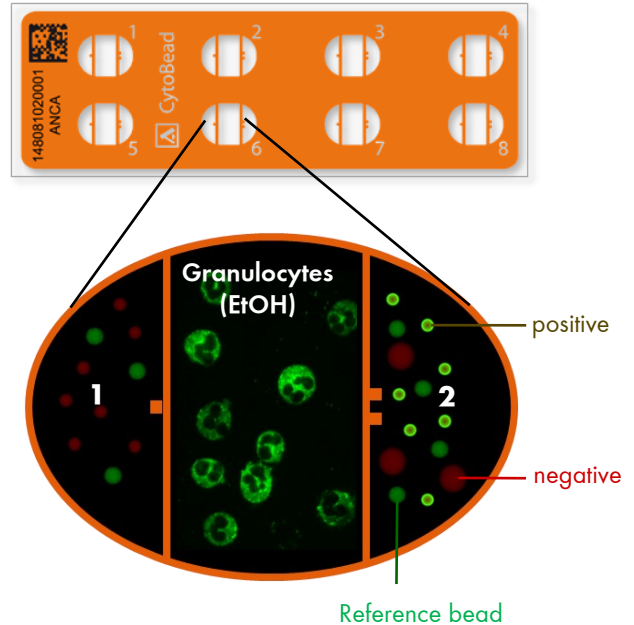
ANCAs (Anti-Neutrophil Cytoplasmic Antibodies) play an important role in the diagnosis of ANCA associated vasculitides (AAV). According to international guidelines ANCA screening is performed using immunofluorescence (IFA) with ethanol-fixed granulocytes, whereby cytoplasmic (cANCA; antigen PR3) and perinuclear (pANCA; antigen MPO) IFA patterns can be differentiated.

Unique combination of ethanol-fixed granulocytes with antigen-coated microbeads

### Advantages of CytoBead<sup>®</sup> ANCA

- **Screening** with standardized ethanol fixed granulocytes
- **Confirmation** of 3 ANCA-specific antigens

## CytoBead<sup>®</sup> ANCA



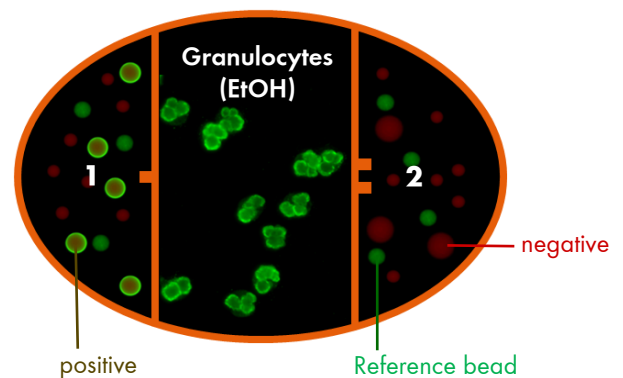
## RPGN (Rapidly Progressive Glomerulo-Nephritis)

Especially indicated in emergency situations

### Advantages of CytoBead<sup>®</sup> RPGN

- **Screening** with standardized ethanol-fixed granulocytes
- **Confirmation** of 3 ANCA + RPGN (dsDNA)-specific antigens

## CytoBead<sup>®</sup> RPGN



## CytoBead<sup>®</sup> ANCA / RPGN

Cell pattern	Compartment	Bead (positive)	Quantification	Antigen	Clinical relevance
negative	1		U/ml	GBM	Goodpasture syndrome
cytoplasmic	2		IU/ml	PR3	Granulomatosis with polyangiitis
perinuclear	2		IU/ml	MPO	Microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis, polyarteritis nodosa

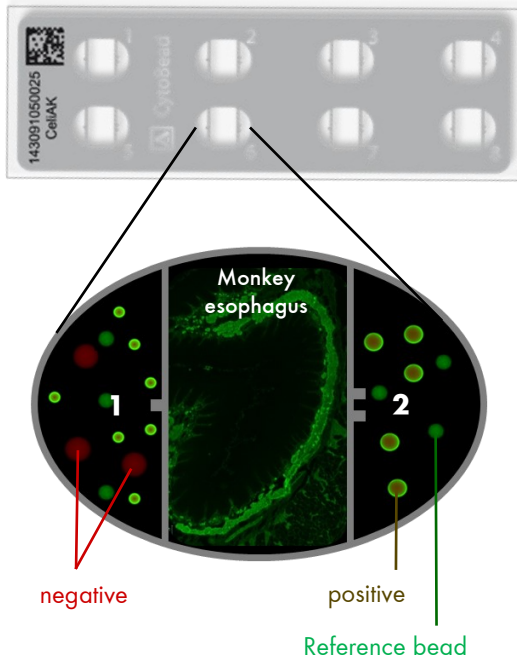
## Additionally in CytoBead<sup>®</sup> RPGN

nuclear	1		IU/ml	dsDNA	Lupus nephritis
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# CytoBead<sup>®</sup> CeliAK

## CytoBead<sup>®</sup> CeliAK



## Fast and easy diagnostics of celiac disease or dermatitis herpetiformis and detection of IgA deficiency

Celiac disease (gluten induced enteropathy) is an intolerance to gluten. This intolerance leads to extended lesions of the mucous membranes, which manifests as a "flat" mucosa. Gliadin, the alcohol-soluble fraction of gluten, is responsible for the emergence of celiac disease. Gliadin induces inflammatory processes in the small intestinal mucosa as part of the humoral and cellular immune processes. The diagnosis of celiac disease is characterized through highly specific autoantibodies against transglutaminase 2 (tissue transglutaminase, tTG) and deamidated gliadin (DG). Endomysial antibodies (EmA) are directed against extracellular tTG. Celiac specific antibodies are usually of IgA class but in patients with IgA deficiency the IgG class is of diagnostic significance.

Unique combination of esophageal tissue with antigen-coated microbeads

### Advantages of CytoBead<sup>®</sup> CeliAK

- **Screening** with standardized monkey esophageal tissue
- **Confirmation** of **3** celiac-specific antigens

## CytoBead<sup>®</sup> CeliAK

Pattern Tissue	Compartment	Bead (positive)	Quantification	Antigen	Clinical relevance
honeycomb pattern of muscularis mucosa	1		U/ml	tTG	Celiac disease, dermatitis herpetiformis
	1		U/ml	DG	
	2		U/ml	Anti-IgA	IgA deficiency

## Simultaneous detection of celiac disease-specific IgA antibodies and total IgA

Grossmann K, Röber N, Hiemann R, Rödiger S, Schierack P, Reinhold D, Laass M.-W., Conrad K, Roggenbuck, D.

Auto Immun Highlights. 2016 Dec, 7(1): 2

## Next-Generation Autoantibody Testing by Combination of Screening and Confirmation – the CytoBead<sup>®</sup> Technology

Sowa M, Hiemann R, Schierack P, Reinhold D, Conrad K, Roggenbuck D.

Clinic Rev Allerg Immunol, DOI 10. 1007/s12016-016-8574-3, ISSN 1080-0549 (2016)

## Second generation analysis of antinuclear antibody (ANA) by combination of screening and confirmatory testing

Scholz J, Grossmann K, Knütter I, Hiemann R, Sowa M, Röber N, Rödiger S, Schierack P, Reinhold D, Bogdanos DP, Meroni PL, Radice A, Conrad K, Roggenbuck D.

Clin Chem Lab Med 2015, 53(12): 1991-2002

## Simultaneous automated screening and confirmatory testing for vasculitis-specific ANCA

Sowa M, Grossmann K, Knütter I, Hiemann R, Röber N, Anderer U, Csernok E, Bogdanos DP, Borghi MO, Meroni PL, Schierack P, Reinhold D, Conrad K, Roggenbuck D.

PLoS One. 2014, 9(9):e107743

## The CytoBead assay - a novel approach of multiparametric autoantibody analysis in the diagnostics of systemic autoimmune diseases

Sowa M, Grossmann K, Scholz J, Röber N, Rödiger S, Schierack P, Conrad K, Roggenbuck D, Hiemann R.

J Med Lab 2014; 38:309-17

# Overview - CytoBead<sup>®</sup> Assays

## Manual assays



Test	Order No.	Determinations
CytoBead <sup>®</sup> ANA	8065	80 (10 x 8)
CytoBead <sup>®</sup> ANA 2	8220	80 (10 x 8)
CytoBead <sup>®</sup> ANCA	8063	48 (6 x 8)
CytoBead <sup>®</sup> CeliAK	8064	48 (6 x 8)
CytoBead <sup>®</sup> RPGN	8066	48 (6 x 8)
CE certified		
CytoBead <sup>®</sup> ANA DFS-70	8260	80 (10 x 8)
For research use only		

## Automated assays for AKLIDES<sup>®</sup> and *akiron*<sup>®</sup> NEO



Test	Order No.	Determinations
AKLIDES <sup>®</sup> CytoBead <sup>®</sup> ANA	4272	80 (10 x 8)
AKLIDES <sup>®</sup> CytoBead <sup>®</sup> ANA 2	4277	80 (10 x 8)
AKLIDES <sup>®</sup> CytoBead <sup>®</sup> ANCA	4270	48 (6 x 8)
AKLIDES <sup>®</sup> CytoBead <sup>®</sup> CeliAK	4271	48 (6 x 8)
AKLIDES <sup>®</sup> CytoBead <sup>®</sup> RPGN	4281	48 (6 x 8)
CE certified		



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