

# Flow2 CAST<sup>®</sup>

## The Routine Friendly Assay for Cellular Allergy Diagnosis

There is more  
to allergy than  
just IgE

Easy detection of Basophil Activation  
for whole blood samples

New Marker combination: CCR3/  
CD63 for easy gating

Time to result down to 60 minutes

New rapid tool for determination of  
drug allergies and hypersensitivities



# Cellular Allergy Diagnosis with CCR3/CD63

## Basophil Activation in Allergy Diagnosis

Allergy is a chronic disease with high morbidity, causing high costs to health systems and has become a significant socio-economic topic. Not all allergies are easy to diagnose: sIgE serologies reach their limits in numerous cases especially when drug hypersensitivities are involved like allergies to antibiotics and sensitivities to anaesthetics and NSAIDs. Thus in vivo tests and provocations are required to determine the origin of the patients' reactions.

In recent years the cellular in vitro allergy tests have become an important addition to allergy diagnosis. Published results confirm the high value of the results concerning sensitivities and specificities (see refs 1-7).

The BÜHLMANN CAST® Assays are at the forefront of this development, as BÜHLMANN is the only company specialized in development and validation of cellular allergy assays. This is also underlined by the fact that BÜHLMANN offers a complete package for allergy diagnosis that includes over 120 standardized allergens.

A Swiss study estimates the incidence of life threatening anaphylaxis caused by allergy at 8-10 cases per 100000. 60% of anaphylactic shocks are caused by Hymenoptera stings, 20% by reactions to drugs and 10% of anaphylactic reactions are caused by food allergies. These allergies are the focus applications of the CAST® Assays. International studies confirm sensitivities between 45 to 90% and specificities of up to 100%, depending on the allergens tested.

## The new Flow2 CAST®

The Flow2 CAST® is the latest development of CAST Assays offered by BÜHLMANN:

### Whole Blood Samples

BÜHLMANN developed the Flow2 CAST with one main goal, being the simplification of the protocol.

Sophisticated adaptations of ion levels for our reagents allow the application of the assay directly with EDTA whole blood samples, strongly simplifying the test procedure, limiting the blood volume required and speeding up the whole assay significantly.

### CCR3 as Basophil Marker

CCR3 is an eotaxin receptor and is constitutively expressed on basophils. CCR3 is detected with a PE-flourescence labeled monoclonal Ab. With the introduction of this development, the gating of basophil cells is significantly easier to accomplish now with the new Flow2 CAST, and contamination of basophils with monocytes can not occur anymore.

### CD63 as Activation Marker

When basophil cells are stimulated *in vitro* by incubation with allergens, resulting CD63 expression on the cell surface is detected with a monoclonal antibody that is FITC-flourescence labeled. CD63 is a reliable and the most published basophil activation marker for allergy detection.

### Additional Stimulation Control

In order to circumvent the potential IgE non responders in the tested populations (estimated 5%), the Flow2 CAST contains FMLP as additional positive control, which acts independently from IgE.

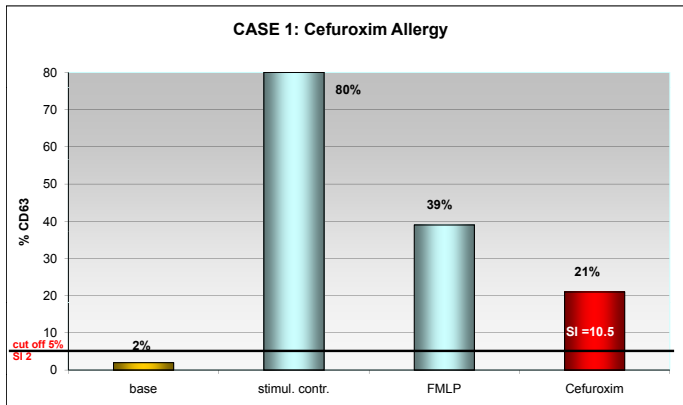
## The Flow2 CAST® New Assets

- Whole blood samples
- No leucocyte isolation
- Easy handling of the samples
- Time to result 1 hour
- Two color assay
- Easy gating and data analysis
- Two independent positive controls: anti-Fc receptor Ab and FMLP

## References

- 1 ENDA Group. Poster at EAACI (2006)
- 2 Gamboa P et al. Clin Exp Allergy 34, 1448-57 (2004)
- 3 Sanz ML et al. Int Arch Allergy Immunol 124, 307-8 (2001)
- 4 Sanz ML et al. Int Arch Allergy Immunol 130, 33-9. (2003)
- 5 Sanz ML et al. Int Arch Allergy Immunol 136, 58-72 (2005)
- 6 Sturm GJ et al. Allergy 59, 1110-7 (2004)
- 7 Torres MJ et al. Allergy 58, 961.72 (2003)

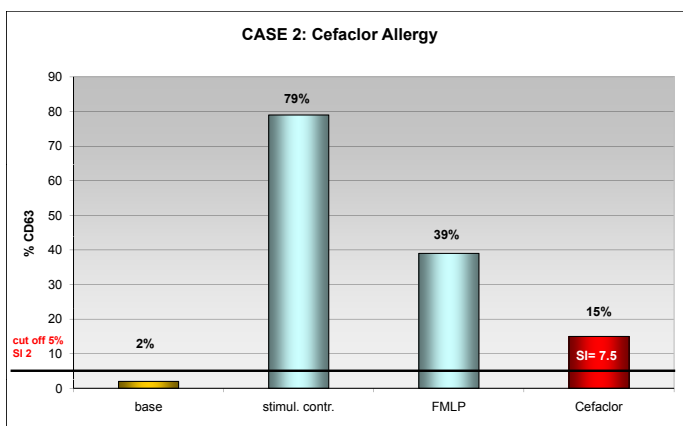
# Case Reports



Female patient, age 61, developed an anaphylactic shock as reaction to Cefuroxim. Skin prick tests resulted positive for Cefuroxim and Ciprofloxacin. Determination of specific IgE serology was negative.

**Flow2 CAST:** In vitro stimulation of the patient's whole blood sample with Cefuroxim BÜHLMANN allergen, gave a clearly positive signal, shown as percentage of CD63 expression on basophil cells (21% and Stimulation Index of 10.5 - cut off: 5% and SI 2). Negative control was below the 5% cut off and both stimulation controls were highly positive.

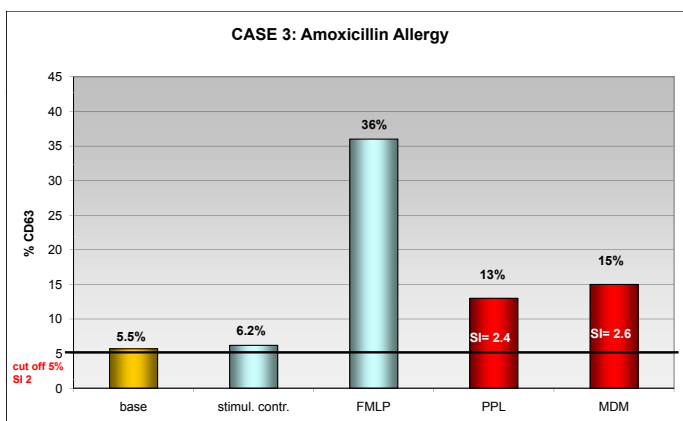
(Data courtesy of Prof. B. Eberlein, TU Munich)



Male patient, age 19, developed an anaphylactic shock as reaction to Cefaclor, eight months before diagnostic tests were performed. Skin tests resulted positive for Cefaclor. Determination of specific IgE serology was negative to Cefaclor.

**Flow2 CAST:** In vitro stimulation of the patient's whole blood sample with Cefaclor resulted in a clearly positive signal, shown as percentage of CD63 expression on basophil cells (15% and Stimulation Index of 7.5 cut off: 5% and SI 2). Negative control was below the 5% cut off and both stimulation controls were highly positive.

(Data courtesy of Prof. A. Romano, Columbus Institute, Rome)

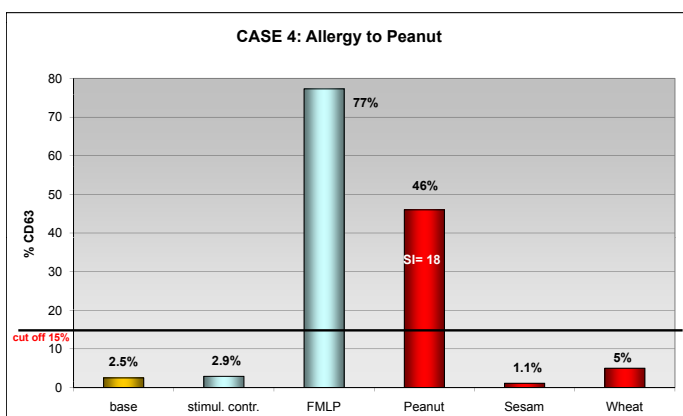


Patient, age 46, developed a serious urticaria as reaction to Amoxicillin in February 2007. Determination of specific IgE serology was negative. Skin tests for PPL, MDM and Amoxicillin were negative.

**Flow2 CAST:** In vitro stimulation of the patient's whole blood sample with BÜHLMANN PPL and MDM allergens - measured together with the culprit allergen as recommended - resulted in clearly positive signals. PPL: 13%, MDM: 15%, Stimulation Indexes were 2.4 and 2.6 (cut off: 5% and SI 2).

Negative control was below the 5% cut off. FMLP as stimulation control was highly positive, showing the advantage of including two independent positive controls in the assay.

(Data courtesy of Prof. M. Blanca, Dr. L. Mayorga, Carlos Haya Hospital, Malaga)



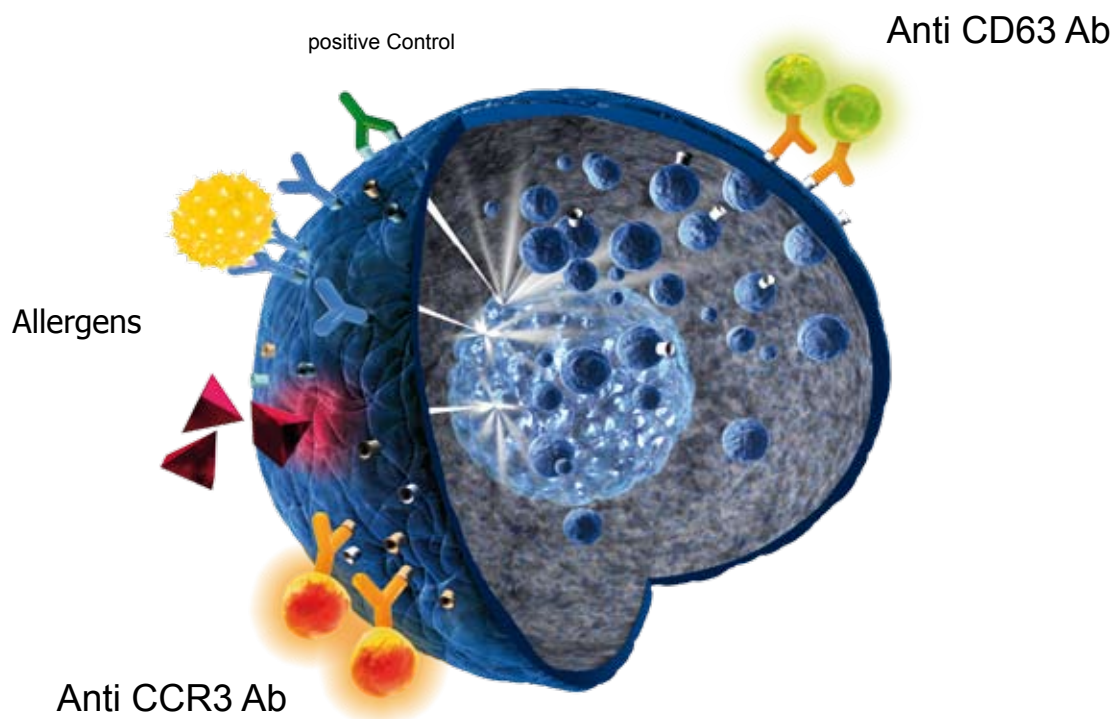
Young male patient, aged 15, with suspected reactions to peanut. Controlled food challenge determined peanut as culprit allergen. Specific IgE serology was positive for peanut, but also for sesame, soy and wheat.

**Flow2 CAST:** In vitro stimulation of the patient's whole blood sample with the BÜHLMANN peanut allergen showed a highly positive signal: 46% and Stimulation Index of 18 (cut off: 15%). Sesame and wheat caused no basophil activation (below 5%), confirming the superior specificity of the Flow2 CAST, compared to the sIgE measurements.

Negative control was below the 15% cut off. FMLP as stimulation control was highly positive.

(Data courtesy of Dr. S. Seneviratne, A. Snorton, St. Mary's Hospital, London)

# Flow2 CAST<sup>®</sup> Cellular Allergy Diagnosis with CCR3/CD63



Easy Quick Reliable

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**Ordering code:**

EK-CAST	192 wells
EK-CAST5	480 wells
FK-BAT	100 tests
FK-BAT2	200 tests

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