



Basophil Activation test for the diagnosis of anaphylaxis



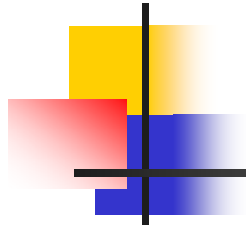
Laboratory tests for type I allergy

- Determination of atopic status: total IgE
- Identification of allergens:
 - skin test (prick, intradermal)
 - Serum specific IgE (RAST, Unicap, ELISA, line blot)
 - Basophil activation
 - Histamine release
 - Sulfidoleukotriene (sLT) release by ELISA (CAST-ELISA)
 - Activation marker CD63 measured by flow cytometry (FLOW-CAST), also called basophil activation test (BAT), flow-cytometric allergen stimulation test (FAST)
 - Challenge tests



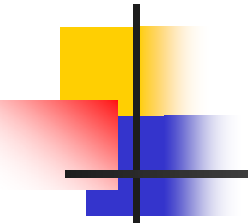
A patient develops anaphylaxis during general anaesthesia

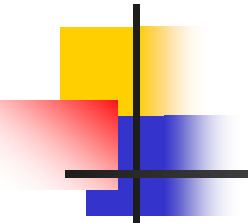
30/10/08



- F/55
- PHx: DM, schizoaffective disorder and on diazepam, haloperidol, danoil, metformin, orphanadin acetate and lisinopril.
- 18/8/2008 OT for genital prolapse
- given Fentanyl 100ug, propofol 150mg and suxamethonium 100mg and developed anaphylaxis

- blood pressure taken at a 5 minute interval in the operating theatre.
- Her blood pressure was taken at 0906 during preoxygenation and intravenous anaesthetic (fentanyl, propofol, suxa) were then injected before 0907.
- Intubation was done smoothly one minute after the injection at 0908. When the endotracheal tube was connected to the anaesthetic machine, no entidal CO2 was detected on bagging.

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- Direct laryngoscopy was done immediately and the tube was seen in between the vocal cords. So the tube was at the right place, but still the tube was pulled out very soon because there was no Entidal CO₂ and the patient began to desaturate.
 - Upon bagging with 100% oxygen via FM, the SpO₂ reading continued to drop.
 - tried to reintubate immediately and at the same time (at 0911) the blood pressure could not be measured.
 - radial pulse not detectable and ephedrine 6mg +6mg was given. The ECG was sinus all along and the heart rate stayed at 104 after the injection of ephedrine.
 - expected it to increase but then after maybe 10 seconds the heart rate dropped quickly to 65.
 - The intubation was done at this time and the patient was again bagged with 100% oxygen and still no entidal CO₂ detected.

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- 0.6mg atropine given. Soon after the injection of atropine, blood pressure was measurable and the reading was 40/17(at 0913) and at the same time Entidal CO2 was detectable and SpO2 95.
 - The systolic blood pressure was around 60-80 mmHg after some intravenous ephedrine and phenylephrine given

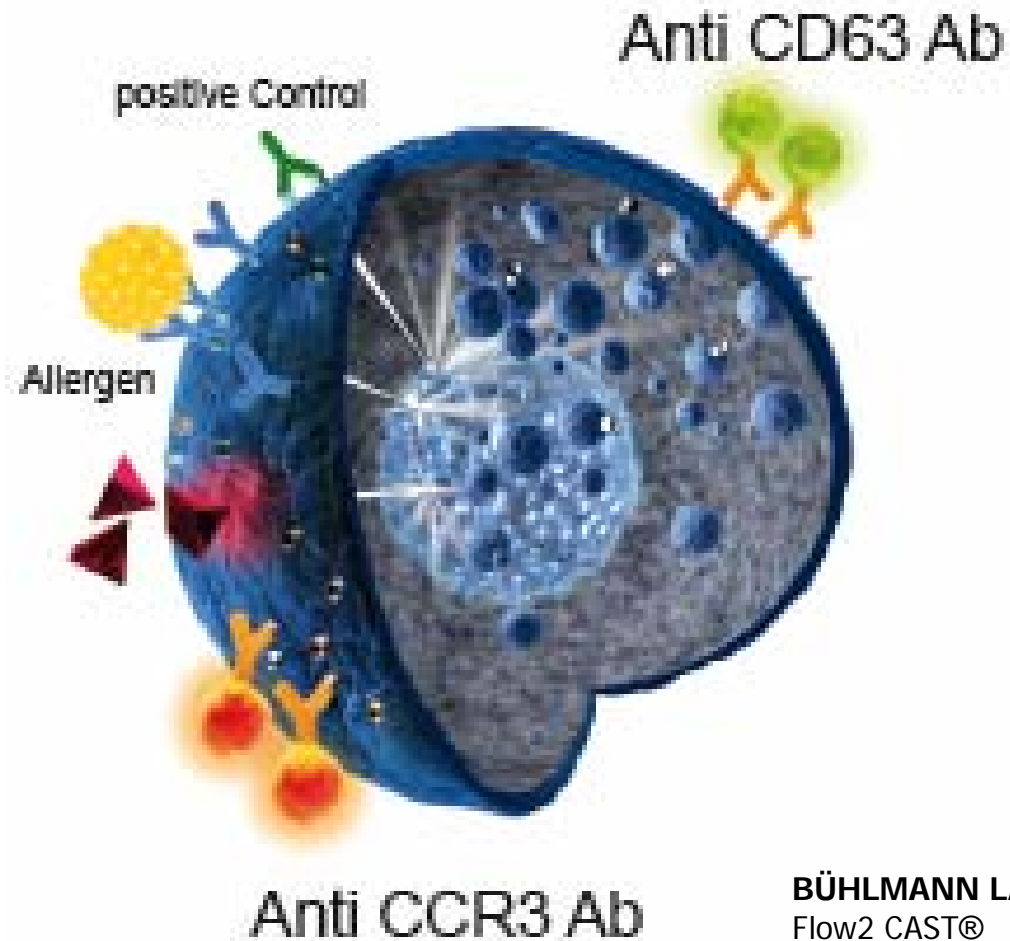
 - Her tryptase result was:
 - Geometric mean 3.8 ug/L, 95 upper percentile: 11.4
 - at 0930 76.3ug/L
 - at 1030 68.8ug/ml
 - at 1500 29.6ug/ml



Anaphylaxis

- Life-threatening causes:
 - Hymenoptera sting 60%
 - Drugs 20%
 - Food 10%
- Drugs
 - Anti-biotics
 - Anaesthetic drugs especially neuromuscular blocking agents
 - analgesics

Basophil activation tests





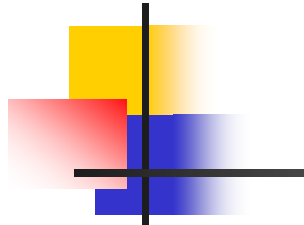
Basophils

- Equivalent of mast cells
- Release histamine and other mediators
 - IgE (anaphylactic)
 - Non-IgE (anaphylactoid e.g. radio-contrast medium)
- Basophilic granules
- <0.2% of peripheral blood leucocytes



Reagents

- Anti-CCR3
 - Eotaxin receptor
 - Constitutively expressed on basophils and eosinophils
 - Alternative: anti-IgE, CD123 (IL-3R α chain), HLA-DR -ve
- Anti-CD63
 - Activation markers
 - 53 kDa
 - If not activated, expressed on inside of vesicle membrane
 - When activated, fused with plasma membrane
 - Not specific, also expressed in monocytes, platelets
- Anti-IgE receptor
 - Against Fc ϵ RI
 - Highly specific
- fMLP
 - Potential IgE non-responders, about 5% of the population
 - Non-specific, non-immunological



INSECTS

BAG2-I1	HONEY BEE VENOM
BAG2-I3	YELLOW JACKET VENOM
BAG2-I4	PAPER WASP VENOM
BAG2-I75	EUROPEAN HORNET

DRUGS

ANTIBIOTICS

BAG2-C1	PENICILLIN G
BAG2-C11	BENZYLPENICILLOYL-POLYLYSIN, PPL
BAG2-C12	BENZYLPENICILLIN +-ACID, MDM
BAG2-C2	PENICILLIN V
BAG2-C3	CEPHALOSPORIN C
BAG2-C31	CEFAMANDOLE
BAG2-C32	CEFAZOLIN
BAG2-C33	CEFUROXIME
BAG2-C61	SULFAMETHOXAZOLE
BAG2-C62	TRIMETHOPRIM
BAG2-C75	TETRACYCLINE
BAG2-C81	CIPROFLOXACIN
BAG2-C203	AMPICILLIN (EK-CAST)
BAG2-C203F	AMPICILLIN (FK-BAT)
BAG2-C204	AMOXICILLIN
BAG2-CRIF	RIFAMPICIN new
BAG2-CCLA	CLARYTHROMYCIN new

ANALGESICS

BAG2-C51	LYS-ASPIRIN
BAG2-C52	DICLOFENAC
BAG2-C53	IBUPROFEN
BAG2-C54	INDOMETHACIN
BAG2-C55	ACETAMINOPHEN
BAG2-C56	MEFENAMIN ACID
BAG2-C57	PHENYLBUTAZONE
BAG2-C58	PROPYPHENAZONE
BAG2-C59	DIPYRONE / METAMIZOLE
BAG2-CNAP	NAPROXEN new

ANESTHETICS/ MYORELAXANTS

BAG2-CATR	ATRAURIUM
BAG2-CLID	LIDOCAINE
BAG2-CMIV	MIVACURIUM
BAG2-CPAN	PANCURONIUM
BAG2-CPRO	PROPOFOL
BAG2-CROC	ROCURIUM
BAG2-CSUX	SUXAMETHONIUM
BAG2-CVEC	VECURIUM

INHALANT

GRASSES

BAG-G2	BERMUDA GRASS
BAG-G3	ORCHARD GRASS
BAG-G5	PERENNIAL RYE GRASS
BAG-G6	TIMOTHY GRASS
BAG-G12	CULTIVATED RYE GRASS

WEEDS

BAG-W1	COMMON RAGWEED
BAG-W6	MUGWORT
BAG-W9	RIBWORT/PLANTAIN
BAG-W19	PARIETARIA OFFICINALIS

TREES

BAG-T3	COMMON BIRCH
BAG-T4	HAZEL
BAG-T7	OAK
BAG-T9	OLIVE

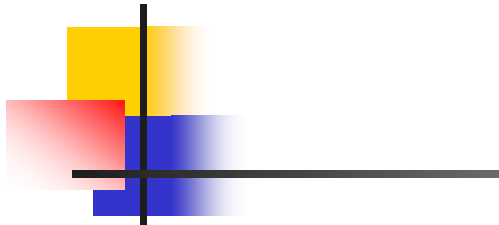
MOLDS

BAG-M1	PENICILLIUM NOTATUM
BAG-M2	CLADOSPORIUM HERBARUM
BAG-M3	ASPERGILLUS FUMIGATUS
BAG-M5	CANDIDA ALBICANS
BAG-M6	ALTERNARIA TENUIS

ANIMALS

BAG-D1	D. PTERONYSSINUS
BAG-D2	D. FARINAE
BAG-D70	ACARUS SIRO
BAG-E1	CAT EPITHELIUM
BAG-E2	DOG EPITHELIUM

BÜHLMANN LABORATORIES AG
Flow2 CAST®



FOOD

Egg

BAG-F1 EGG WHITE

BAG-F75 EGG YOLK

MILK/ MILK PRODUCTS

BAG-F2 MILK (P575, DEFATTED)

BAG-F76 ALPHA-LACTALBUMIN

BAG-F77 BETA-LACTOGLOBULIN

BAG-F78 CASEIN

FISH & MEAT

BAG-F3 CODFISH

BAG-F23 CRAB new

BAG-F24 SHRIMP new

BAG-F258 SQUID new

BAG-F290 OYSTER

BAG-P4 ANISAKIS

BAG-F26 PORK

BAG-F27 BEEF

FRUITS/LEGUMES

BAG-F25 TOMATO

BAG-F31 CARROT

BAG-F95 PEACH new

BAG-F85 CELERY

SEEDS/ BEANS/ NUTS

BAG-F10 SESAME

BAG-F13 PEANUT

BAG-F14 SOYBEAN

BAG-F17 HAZELNUT

BAG-F20 ALMOND new

BAG-F202 CASHEW NUT new

CEREALES

BAG-F4 WHEAT

BAG-F5 RYE FLOUR

BAG-F6 BARLEY FLOUR

BAG-F7 OAT FLOUR

BAG-F8 MAIZE new

BAG-F9 RICE new

BAG-F45 BAKER'S YEAST

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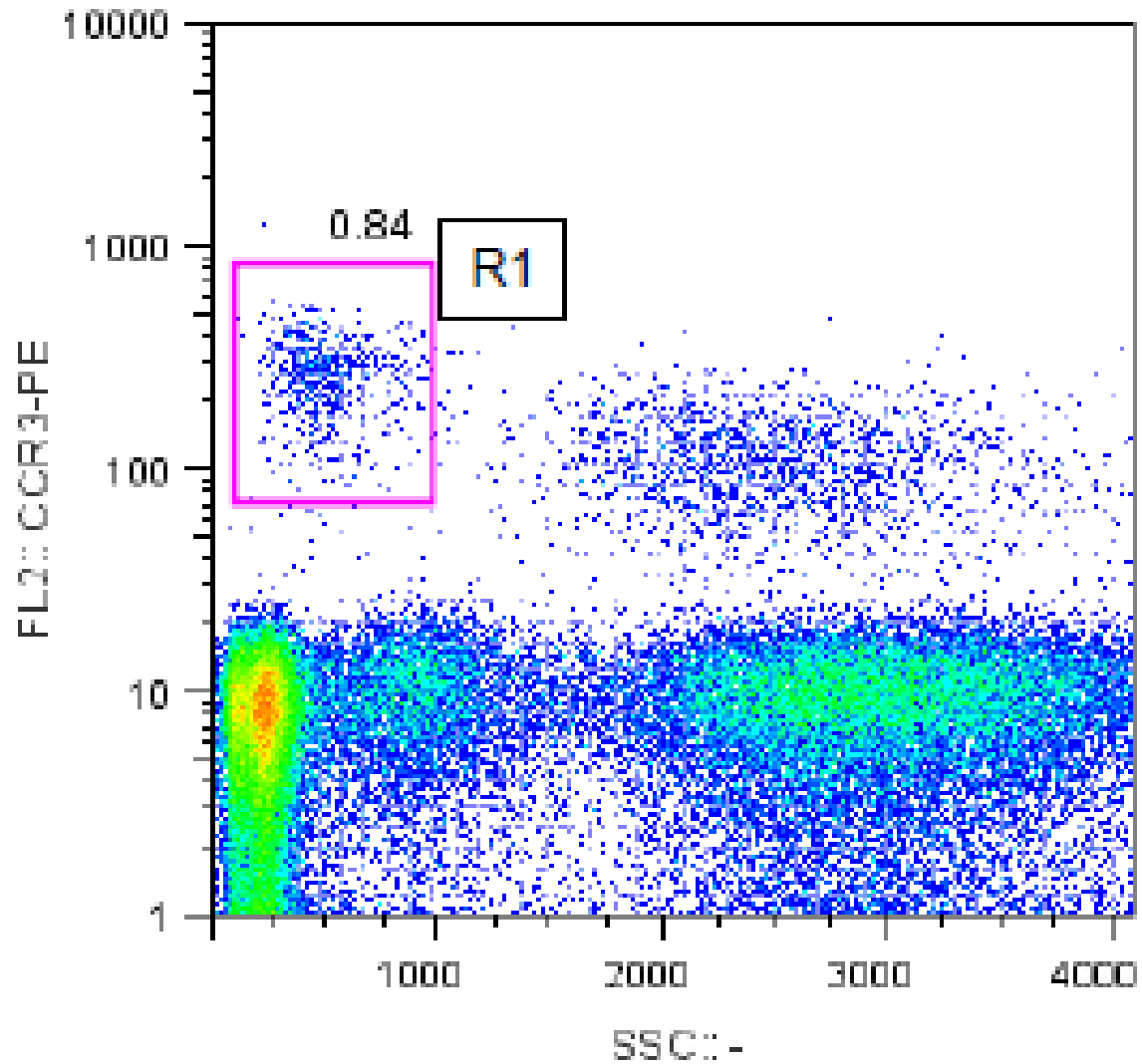
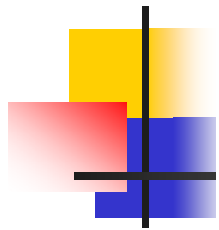
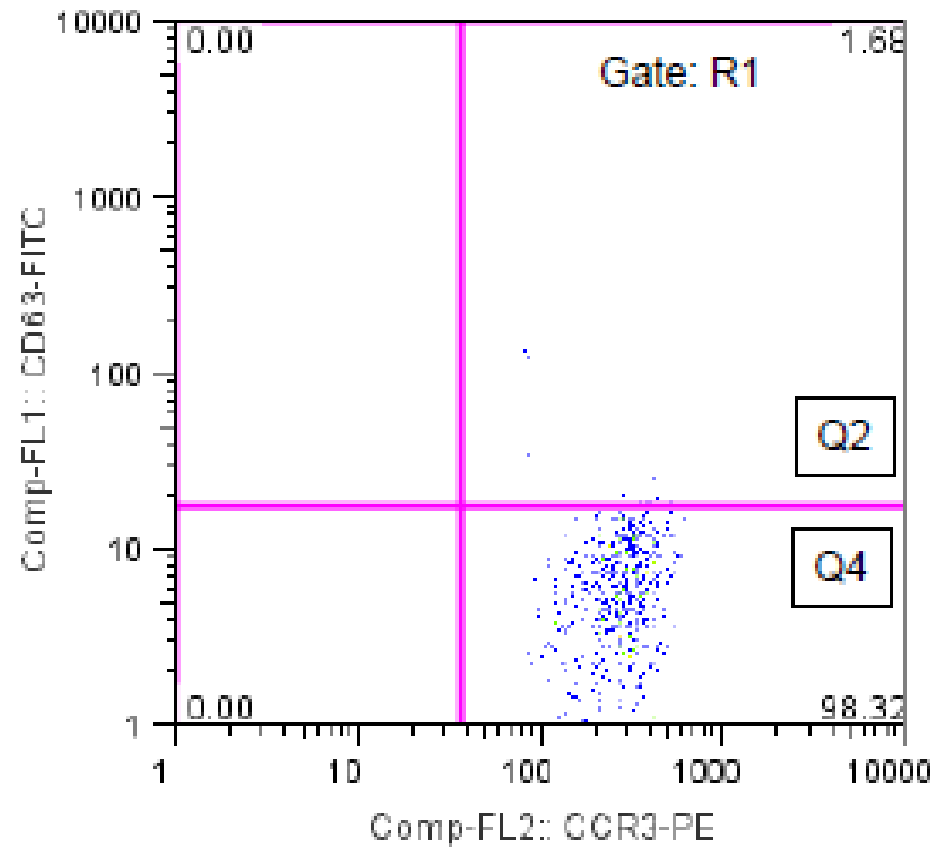
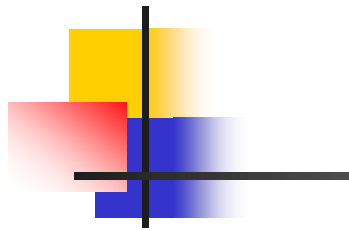
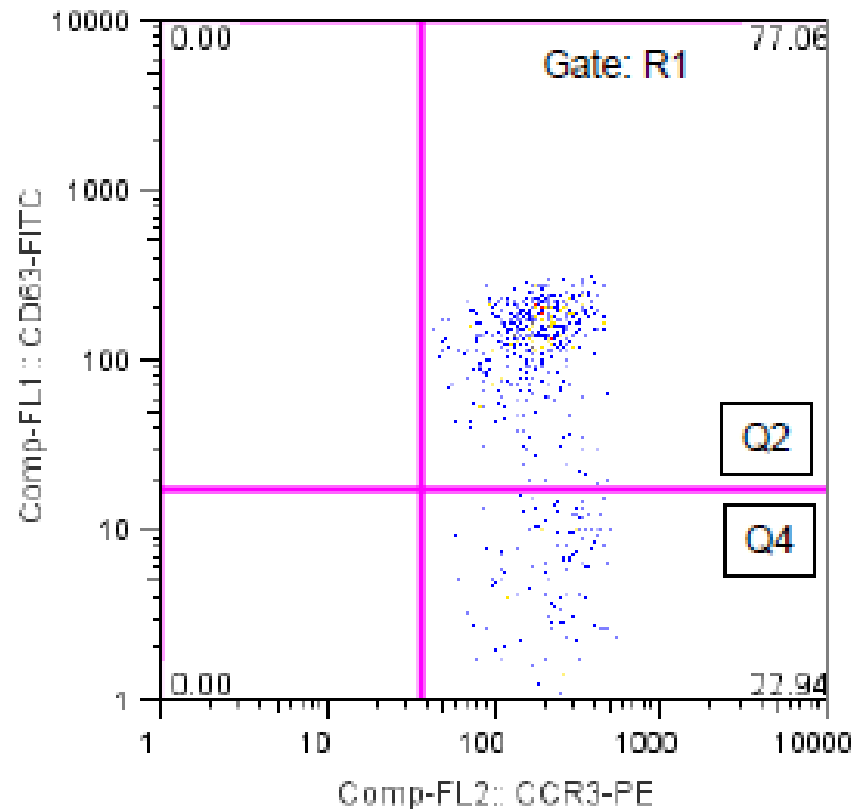
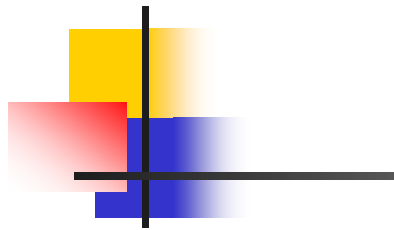


Figure 2: Selection of basophilic cells CCR3^{POS} / SSC^{LOW}



Gated Region	Count (n=)	%
Total	78251	100.0
R1	655	0.8
Q2 (CD63 ^{pos})	11	1.7
Q4 (CD63 ^{neg})	644	98.3

Figure 3: Patient Background (PB) with STB only



Gated Region	Count (n=)	%
Total	72916	100.0
R1	606	0.8
Q2 (CD63 ^{pos})	467	77.1
Q4 (CD63 ^{neg})	139	22.9

Figure 4: Stimulation Control (STCON)



Basophil activation tests

- *In vitro* form of skin test
- Advantages:
 - Safe
 - Specific (90-100%)
- Disadvantages:
 - Variable and generally low sensitivity (50-90%)
 - Expensive
 - Non-responders
 - clinical value less well studied
- At present only for anaphylaxis due to anaesthetic drugs in QMH