Autoimmun Diagnostika GmbH
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Reverse Hybridisation in modern diagnostics

lots of information by one reaction through multiplexing and highly sensitive detection in a standardized and easy to perform procedure

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1. Introduction of AID
2. Method
3. Application TB and KRAS
4. QC and automatisation
5. Summary

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Short company profile:

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- Founded by Dr. Schoellhorn in 1989 located in Straßberg, South-West Germany
- Private owned company
- Certified according to DIN EN ISO 13485 and DIN EN ISO 9001
- Focus on developing and manufacturing of IVDËs and Reader System for evaluation as well as selling this products worldwide
- Molecluarbiological testsystems for human genetics , infection diseases and antibiotic resistance
- T-cell based assay to monitore immunreaction after transplantation

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AID and Arian Gene Gostar

Since 2009 AID maintains a successful partnership with Arian Gene Gostar to provide the products in Iran market.

Based on our philosophy the partnership with Arian Gene Gostar is signed by offering products, support and service to satisfy the needings of labs and safety to patients.

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Reverse Hybridisation procedure

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DNA extraction with any commercially available kit

Standard PCR with biotinylated primers

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Reverse Hybridisation procedure

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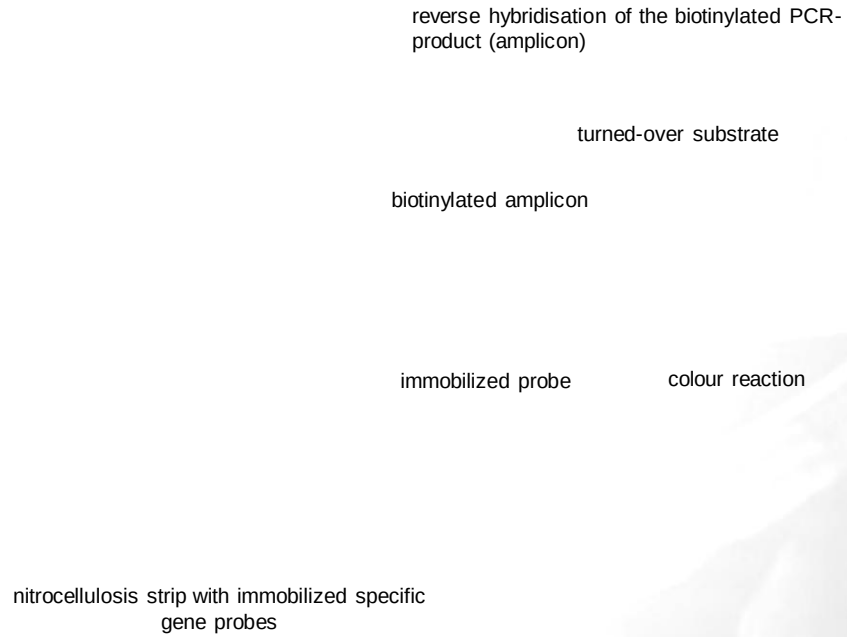
Hybridisation of the PCR-products to strips precoated with oligonucleotide probes specific for the pathogens or polymorphisms



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Characteristics

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Highly specific washing procedure
- only hybrids with 100 % complementary sequences survive

Detection is subsequently achieved by the sequential addition of streptavidin-coupled alkaline phosphatase and the substrate BCIP/NBT to give a coloured product

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Reverse hybridisation kits (infectious diseases):

- ü Sexual transmitted diseases (STD)
- ü Community acquired pneumonia bacterial
- ü Community acquired pneumonia viral
- ü Community acquired pneumonia resistance
- ü Multiple-resistant Staphylococcus aureus (MRSA combi)
- ü Extended spectrum Beta-Lactamase (ESBL)
- ü Mycobacterium tuberculosis complex and TB-resistance (MDR/XDR)
- ü Human Papilloma Virus Screening and Typing
- ü Bordetella pertussis
- ü Periodontisis incl. IL-1 and DR4

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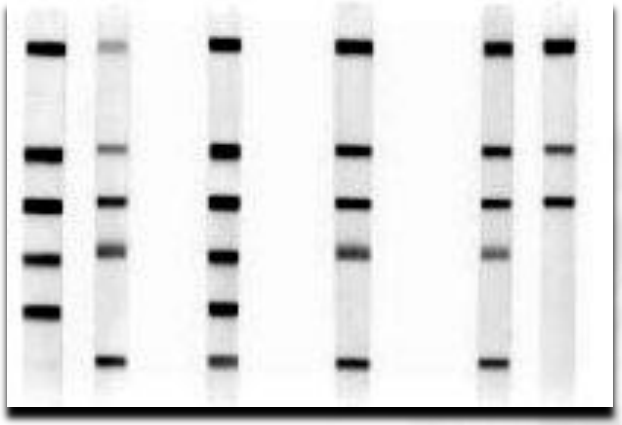

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Immunology:

- ü Morbus Bechterew, HLA B*27 and CYP2D6*4
- ü Rheumatoid arthritis, Shared Epitop HLA DRB1
- ü Celiac Disease, HLA DQ2, DQ8 and DR4

Pharmacogenetics:

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- ü Cytochrome P450 CYP2C19*2
- ü Cytochrome P450 CYP2 *2 & *3, VKORC1
- ü Glutathione S-Transferase M1, T1 and P1
- ü N-acetyltransferase 2 (NAT2)

Haematology:

- ü Factor V Leiden and FII Prothrombin
- ü Factor V Leiden, Prothrombin and MTHFR
- ü Methylentetrahydrofolat reductase (MTHFR)
- ü Hereditary hemochromatosis C282Y, H63D

Metabolism:

- ü Apolipoprotein E and B-100
- ü Osteoporosis risk factors
- ü Lactose intolerance
- ü Hereditary fructose intolerance (HFI)

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Modular design of TB Resistance:

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1. TB Resistance Modul Isoniazid / Rifampicin

- From culture (from direct material is in evaluation)
- Detection of Isoniazid inhA and katG wildtype and mutations
- Detection of Rifampicin rpoB513 ç516, 522 ç526, 529 -533 and the mutations rpoB516, 526, 531.

If there is no mutation (resistance) detected, therapy with standard antibiotic , if there is one mutation detected go further with:

2. TB Resistance Moduls Aminoglycosid and Fluorochinolon

- The Aminoglykosid Modul detect Streptomycin and Kanamycin / Caperomycin wildtypes and mutations.
- The Flurochinolon modul detect the common mutations of gyrA and Ethambutol.

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TB Resistance Module Isoniazid/Rifampicin (RDB2185)

- Conjugate control
- Amplification control
- *Mycobacterium* universal control
- *Mycobacterium tuberculosis* complex
- Isoniazid wild (inhA -16, -15, -8)
- Isoniazid mut (inhA -16, -15, -8)
- Isoniazid wild (katG 315)
- Isoniazid mut (katG 315)
- Rifampicin wild (rpoB 513 - 516)
- Rifampicin mut (rpoB 516)
- Rifampicin wild (rpoB 522 - 526)
- Rifampicin mut (rpoB 526)
- Rifampicin wild (rpoB 529 - 533)
- Rifampicin mut (rpoB 531)

TB Resistance Module Aminoglycosides (RDB2184)

- Conjugate control
- Amplification control
- *Mycobacterium* universal control
- *Mycobacterium tuberculosis* complex
- KAN/AMK wt (rrs 1400/1401)
- KAN/AMK mut (rrs A1400G)
- KAN/AMK mut (rrs C1401T)
- KAN/AMK/CAP wt (rrs 1483)
- KAN/AMK/CAP mut (rrs G1483C/T)
- Streptomycin wt (rpsL43)
- Streptomycin mut (rpsL A43G)
- Streptomycin wt (rpsL 88)
- Streptomycin mut (rpsL A88G)
- Streptomycin mut (rpsL A88C)
- Streptomycin wt (rrs 522-526)
- Streptomycin mut (rrs C522T)
- Streptomycin mut (rrs A523C)
- Streptomycin mut (rrs G524C)
- Streptomycin mut (rrs C526T)

TB Resistance Module Fluoroquinolones (RDB2187)

- Conjugate control
- Amplification control
- *Mycobacterium* universal control
- *Mycobacterium tuberculosis* complex
- Fluoroquinolone wt (GyrA 90, 91, 94)
- Fluoroquinolone mut (GyrA A90V)
- Fluoroquinolone mut (GyrA S91P)
- Fluoroquinolone mut (GyrA D94A)
- Fluoroquinolone mut (GyrA D94N)
- Fluoroquinolone mut (GyrA D94Y)
- Fluoroquinolone mut (GyrA D94G)
- Ethambutol wt (embB 306)
- Ethambutol mut (embB M306V ATG/GTT)
- Ethambutol mut (embB M306I ATG/ATA)
- Ethambutol mut (embB M306I ATG/ATC)
- Ethambutol mut (embB M306I ATG/ATT))

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Universität Zürich[UZH]

Institut für Medizinische Mikrobiologie

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Evaluation of the TB resistance line probe assay

-the most prevalent mutations confering resistance to isoniazid, rifampicin, streptomycin, kanamycin, amikacin, capreomycin, fluoroquinolones and ethambutol

- Verified all probes on the line probe assay; 100% accurate performance
- Clinical *M. tuberculosis* isolates from a low endemic area (Switzerland, n=110) and a high endemic area (South Africa, n=67)

-The line probe assay was evaluated against a series of clinical *M. tuberculosis* strains from Switzerland and South Africa
- Mutations were detected with a 100% accuracy

- Direct screening of smear positive respiratory and non-respiratory clinical specimens
- 98 clinical specimens demonstrated test interpretability > 95%.

- No false positive signals observed

- TB resistance line probe assay is an accurate and easy to use assay, which we have implemented in the routine diagnostics

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GuidoBloemberg,Universityof Zurich2013

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KRAS / BRAF

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Epidemial Growth Factor Receptor (EGFR) was recently recognized as important target of new antibody based therapies against cancer.

Only a part of the cancer patient benefit form this therapy. Reasons for this resistance are mutation on the KRAS and BRAF genes.

Analysis of mutation status of KRAS and BRAF is a very helpful tool for selection of an appropriate therapy.

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KRAS detection of mutation on codon 12, 13 and 61

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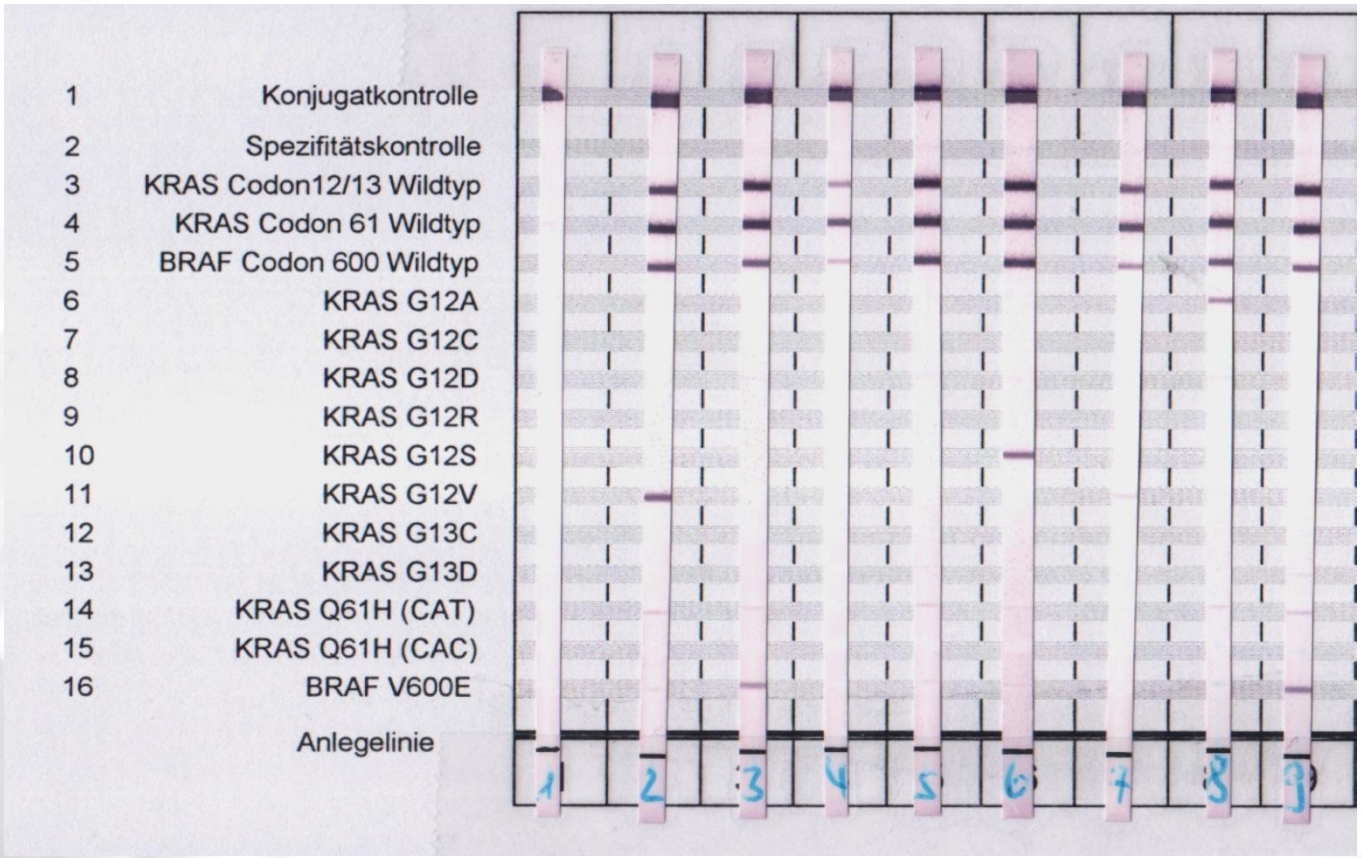

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For in house validation the GenID KRAS assay was processed with a total of 85 patient specimens from seven different pools. Additionally 26 samples from interlaboratory tests of two different suppliers (RfB, INSTAND e.V.) had been analysed.

For external validation the kit was sent to five different laboratories. There, 78 samples of patient specimens or interlaboratory tests (QuIP) had been processed.

Total number of specimens:	189	
true positive:	98	
true negative:	90	
false positive:	0	
false negative:	1	
Sensitivity:	98/(98+1) = 0,989	98,9%
Specificity:	90/(90+0) = 1,000	100%

When a heterogeneous specimen was used, sensitivity of GenID KRAS-Kit was better than 5% tumour cells in wild type cells.
In comparison sensitivity was at least as equal as with reference kits.

Reference kits/methods:
Viennalab (PCR + reverse Hybr. on strip)
Chipron (PCR + reverse Hybr. on chip)
TIBMolBiol (realTime PCR)
Sequencing

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Control zones:

Conjugate control:

Indicates the performance of the hybridisation reaction and must be developed.

Specificity control:

Checks the stringency of the reaction and should never be developed.

Sensitivity control:

Documents the optimal sensitivity of the reaction and should always be developed.

Reaction zones (variable):

These DNA zones are variable in number on each AID Kit. Depending on the genotyp of the patient or the type of infection some zones are developed, while some are not (In the example there are two zones, a wild type zone and a mutant or polymorphic zone).

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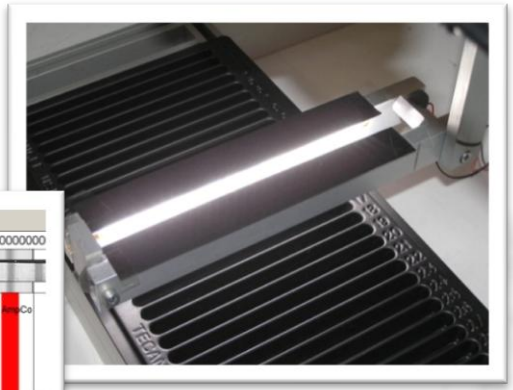
Systems for high throughput:



DNA Extraction with Maxwell 16 and GoTaq from Promega

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Summary

- Fast, accurate, reproducible
- Highly sensitive and specific
- Low-, medium- and high-throughput applications
- Low cost method with standard lab equipment
- Multiplex application offer lots of information on one strip

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Thank you for your attention