



## Introduction to 3D immunofiltration technology

### AREA OF APPLICATION

3D immunofiltration (3D-IF) technology combines elements from affinity chromatography and the principles of immunoassays. Receptors (such as antibodies, antigens, streptavidin, DNA) are immobilized on porous 3-dimensional filters. Subsequently the following reaction steps are carried out in a flow through the filters: Analyte binding, tracer binding, substrate binding and washing steps. This procedure offers the following fundamental advantages:

- Analyte enrichment
- Short assay time (5-15 minutes)
- Precalibration of the assay

The basic idea is to use miniature flow-through columns as depicted in Fig. 1 and 2. Combining this technique with poly-HRP technology allows for ultrasensitive rapid tests. The technology may also be combined with other detection methods (fluorometry, luminometry, magnetic particles etc), or be used for sample extraction.

### THE STRUCTURES OF 3-DIMENSIONAL FILTERS

The key components of the 3D-IF technology are the 3-dimensional filter elements. They are made of polymer sinter or fibrous materials. Surface treatment ensures definite immobilization of proteins and other receptors. The size of the pores can be chosen within a range of approx. 5 to 100µm (Fig. 3). Standard materials based upon polyethylene, have an average pore size of about 20µm with approximately 50% pores. A typical 5\*5mm (h\*d) cylindrical filter -such as those used in the Senova minicolumns (Fig. 2)- has an inner surface of around 40 cm<sup>2</sup>.

### DETECTION METHODS

The 3D filter elements can be combined in numerous detection procedures. Quantification can be performed right on the filter or after elution in the eluate. Senova offers rapid ultrasensitive test Kits under the ABICAP® trademark. These tests are based on 3D-IF technology combined with photometric detection. For ultrasensitive rapid tests Poly-HRP tracers (please refer to our brochure "Introduction to poly-HRP technology") are used in combination with a precipitating enzyme substrate (TMB).

Alternatively dye particle conjugates DSC (Dye Suspensoid Conjugate) can be applied for standard applications. In both cases, the detection takes place directly on the filter by an optical measurement according to Fig. 4. This type of optics is integrated in a small column photometer as shown in Fig. 5. Alternatively the dyes can be eluted and conventional microplates can be used for the quantification of the eluates.

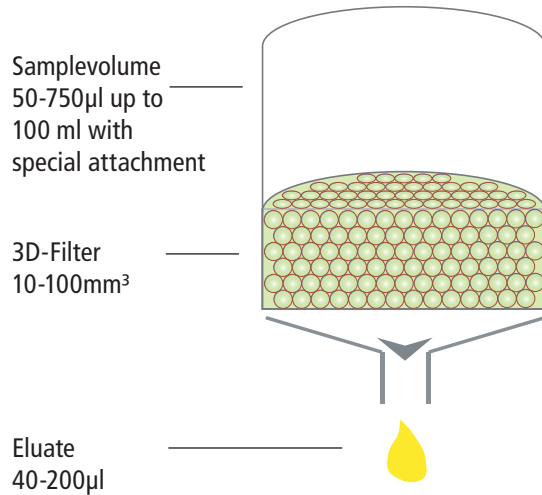


Fig. 1: Principle of 3D-IFA technology



Fig. 2: Mini-columns

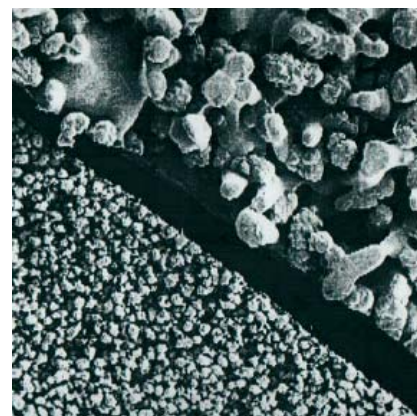


Fig. 3: Typical structures of 3D-Immuno filters (5µm and 100µm)

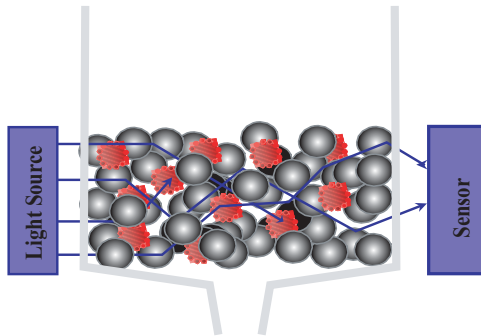


Fig. 4: Detection of dyes on 3D filters using light diffusion measurement.



Fig. 5: Hand-held device for photometric detection of Senova mini columns

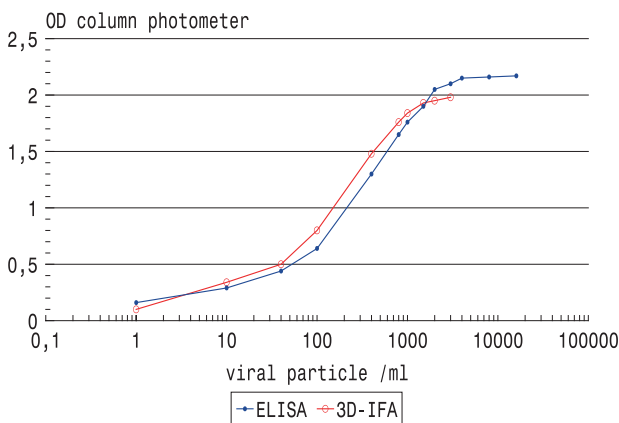


Fig. 6: Calibration curve for the detection of EBOLA viruses; poly-HRP40 ELISA in comparison to the corresponding flow-through immunoassay based on 3D-IF and poly-HRP technology.

(Data acc. to A.Lucht et al.; Bundeswehr Institut für Mikrobiologie, München)

### ABICAP® RAPID TESTS

ABICAP®s are column-based rapid tests with assay times of between 5 and 15 minutes. ABICAP® shows sensitivity data similar to other laboratory procedures (such as ELISA). Fig. 6 shows one example: The reference system displayed here is an ultrasensitive ELISA based on poly-HRP.

If the enrichment effects of the column technology are utilized, detection limits can be pushed further. In general it is possible to transfer established immunoassay reagents to the 3D-IF format to achieve better sensitivity.

Senova offers the corresponding development service (custom development). Table 2 shows a selection of assays in which the process was successfully deployed. The analyte spectrum is wide, ranging from low molecular substances to proteins, viral particles and entire microorganisms.

3D-IF assay protocols are comparable to ELISA protocols. However assay times are considerably shorter. Fig. 7 shows a typical protocol (determination of prostate-specific antigens) Fig. 8, 9 and Table 1 show the results.

### ACCESSORIES

For the convenient handling of ABICAP columns Senova offers racks in the standard 8x12 format. For detection two types of photometers are available. In addition, Senova provides all the reagents needed, from poly-HRP, DSC tracers to ready-to-use substrate solutions and buffers.

### CUSTOM DEVELOPMENT

Senova offers its years of experience with the 3D-IF assay technology within custom development services. Custom development may start from an existing ELISA or include reagent development. A broad portfolio of antibodies, antigens and standards is available. New antigens and antibodies can be developed if requested.

Custom development follows the project scheme depicted in Fig. 10. We will make an individual offering based upon the initial situation, the technical demands and the required project targets.

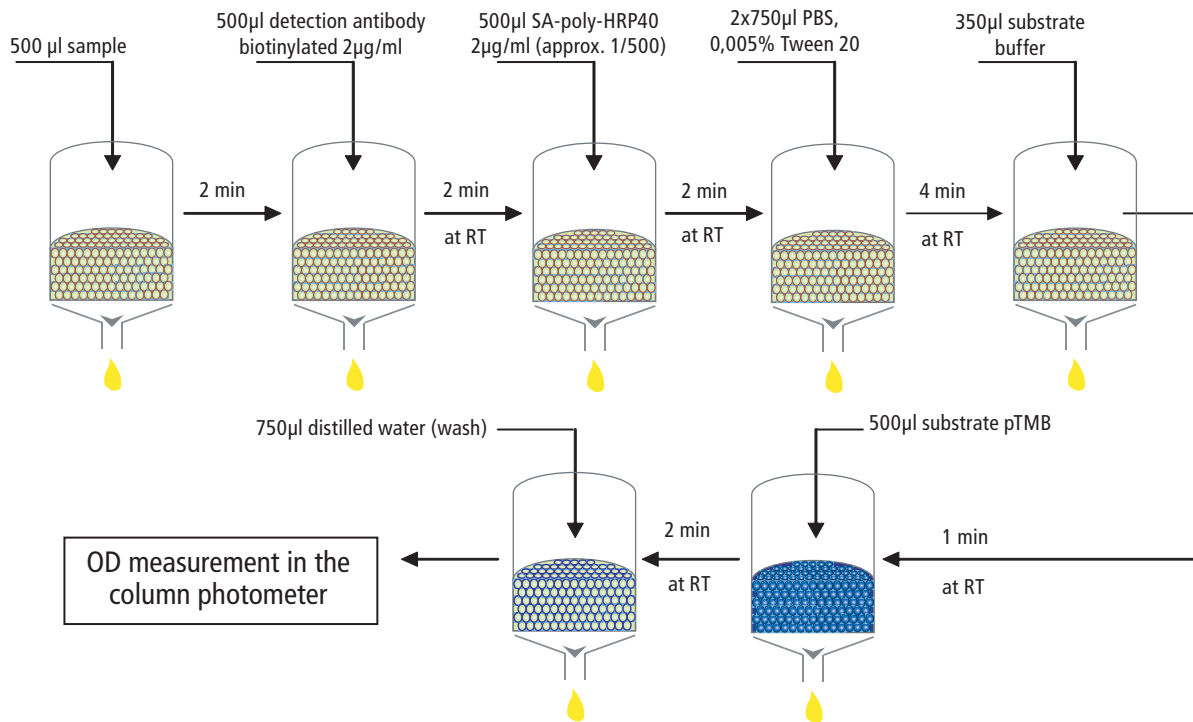


Fig. 7: 3D-IF assay used for PSA

	Mean value [ng/ml]	Intra-Assay-VK [%OD]	Intra-Assay-VK [%concentration]
Sample 1	0,8	12,2	10,1
Sample 2	2,7	6,3	8,8
Sample 3	6,8	6,0	10,7
Sample 4	15	5,5	16,8

Table 1: Intra-assay variation coefficient of the 3D-IF assay for PSA determination (n=12)

Analyte	Type of analyte	Area of application
Prostate specific antigen	Serumprotein	Marker for prostate cancer
C3a /C5a	Serumproteine	septic shock/complement activation
CRP	Serumprotein	Inflammation marker/cardiovasc.marker
IgE	Serumprotein	Allergy
anti tetanus	Specific IgG	Serology, vaccination
anti HIV	Specific IgG/IgM	Serology
anti borrelia	Specific IgG/IgM	Serology, tick infection
Adenovirus	Virus	Stool diagnostics
Rotavirus	Virus	Stool diagnostics
Ebolavirus	Virus	Detection in body fluids
Influenza A/B	Virus	Detection in nasal secretion
Hepes Simplex Virus	Virus	Detection in tear fluid
Coxiella	Mikroorganism	Detection in envir./ patient samples
F.tularensis	Mikroorganism/ LPS	Detection in envir./ patient samples
Legionella	Mikroorganism	Detection in water samples
Cotinin	Nikotinmetabolites	Detection in urine of smokers

Table 2: Selection of 3D-IF assays using poly-HRP detection

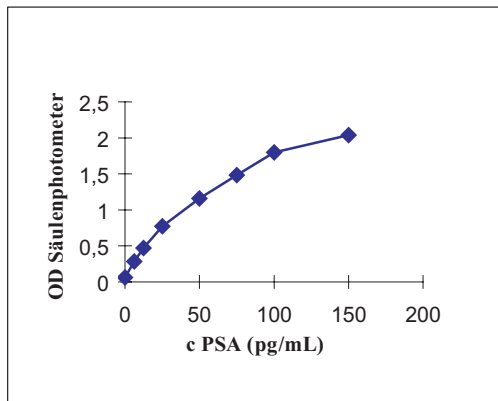


Fig. 8: Typical 3D-IFA standard curve for, e.g. PSA

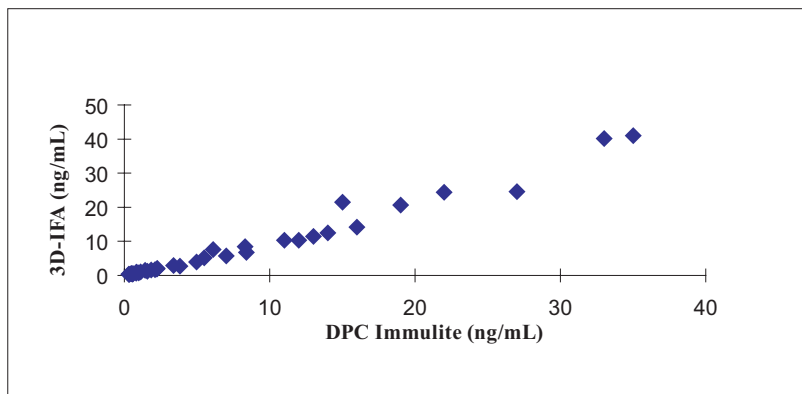
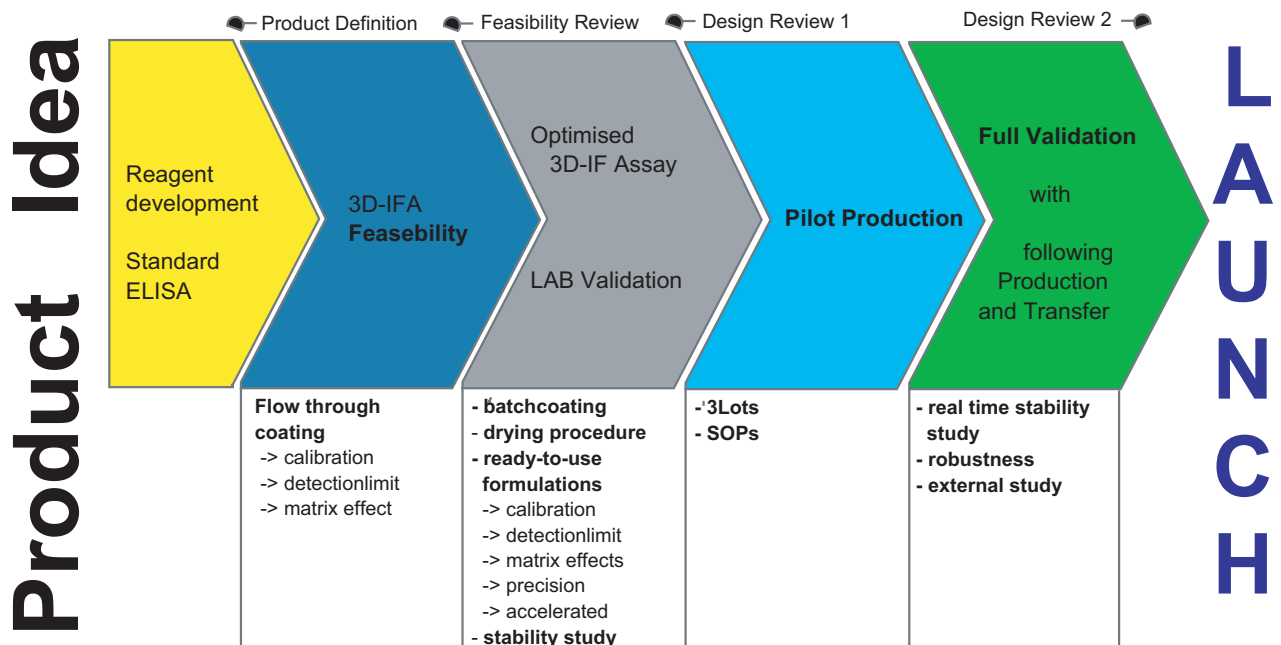


Fig. 9: Correlation between commercial ELISA (DPC Immulite) and 3D-IFA for determining PSA



## Notes

