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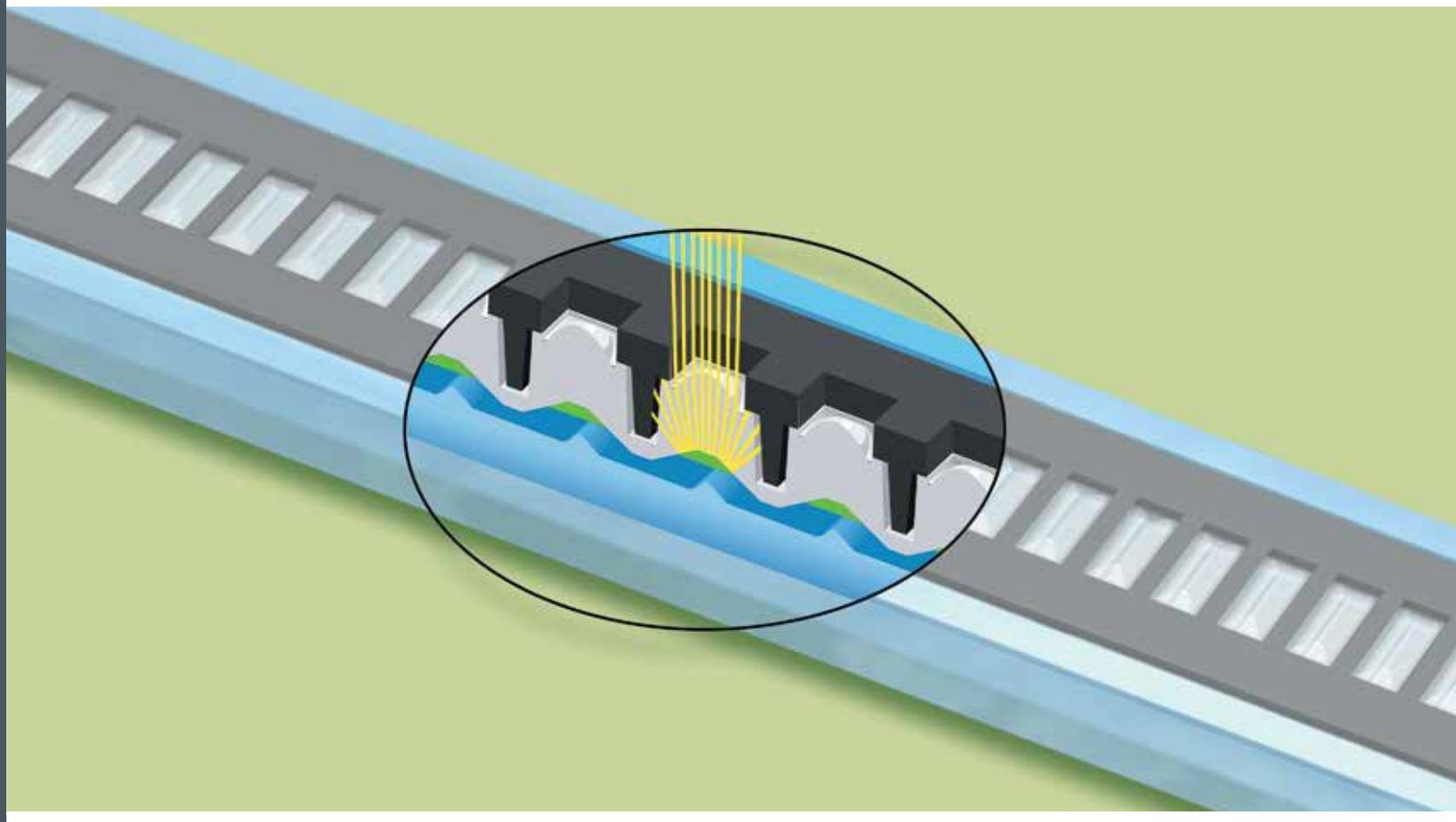

OPTIGEN®

A New Technology for the *In Vitro* Determination Allergen-Specific IgE Levels in Patient Serum

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CONTENTS

I Introduction

The use of *in vitro* diagnostic methods for the determination of allergen-specific IgE in human serum has been widely used in the management of allergic disease. Hitachi Chemical Diagnostics, Inc. (HCD) has been a leader of the panel approach for allergy diagnosis with the use of the CLA® Allergen-specific IgE Assay. HCD has developed a new technology, OPTIGEN®, which allows for multiple allergen testing with a significant reduction in patient serum volume and delivers results in a few hours. The technology consists of a new device that is backwards compatible with all current instrumentation in the field and maintains the multiple allergen format of panel testing. The objective of this monograph is to describe this new technology.

II The Device

The new device consists of three injected molded parts; a pette body, a coverslip and a partition. The pette body contains a serum channel for fluid flow. The coverslip contains wells on the channel side for the binding of allergens and lenslets on the outer side for light collimation. The partition separates the individual wells for light collation. The three parts are assembled to create an enclosed device **(Figure 1)**.

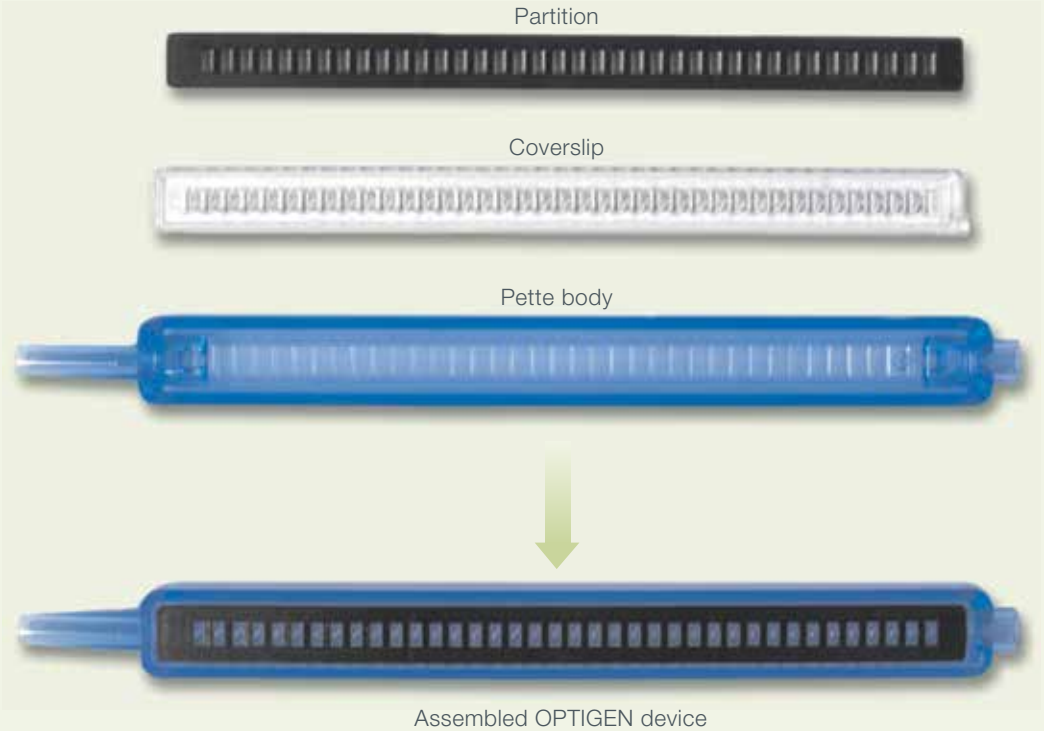

Fig. 1 | OPTIGEN Components and Assembled Device

The internal design of the new device was developed with the aid of Computational Fluid Dynamics (CFD) models and computer simulations. It consists of an array of allergen wells placed in the channel side of the coverslip, coupled with an array of corresponding protrusions on the internal side of the pette body which enhances the washing ability and fluidics of the device **(Figure 2)**.

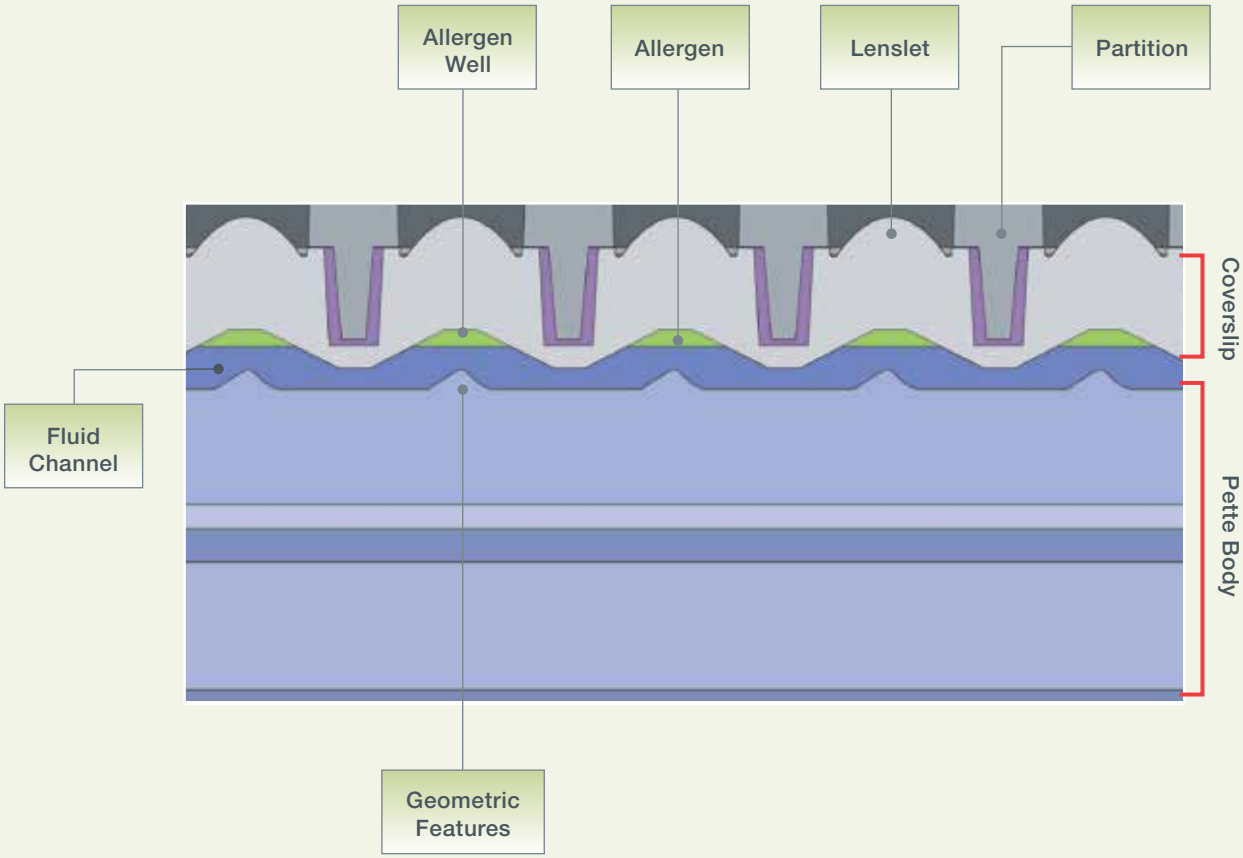

Fig. 2 | Cross-Section of the OPTIGEN Device

The lenslets, designed with the aid of optics computer modeling, are on the opposite side of the allergen well in the coverslip and collimate the light output **(Figures 3 and 4)**.

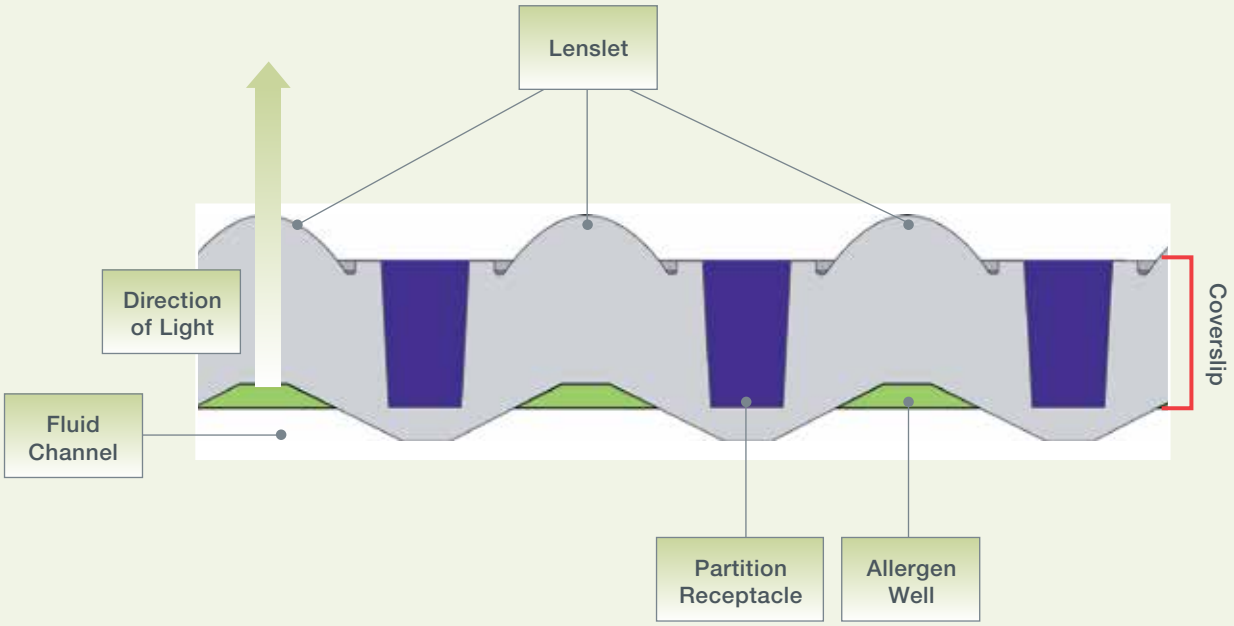

Fig. 3 | Cross-section of the Coverslip for OPTIGEN

The opaque partition is inserted in between the wells to block the light emission from adjacent wells **(Figure 4)**.

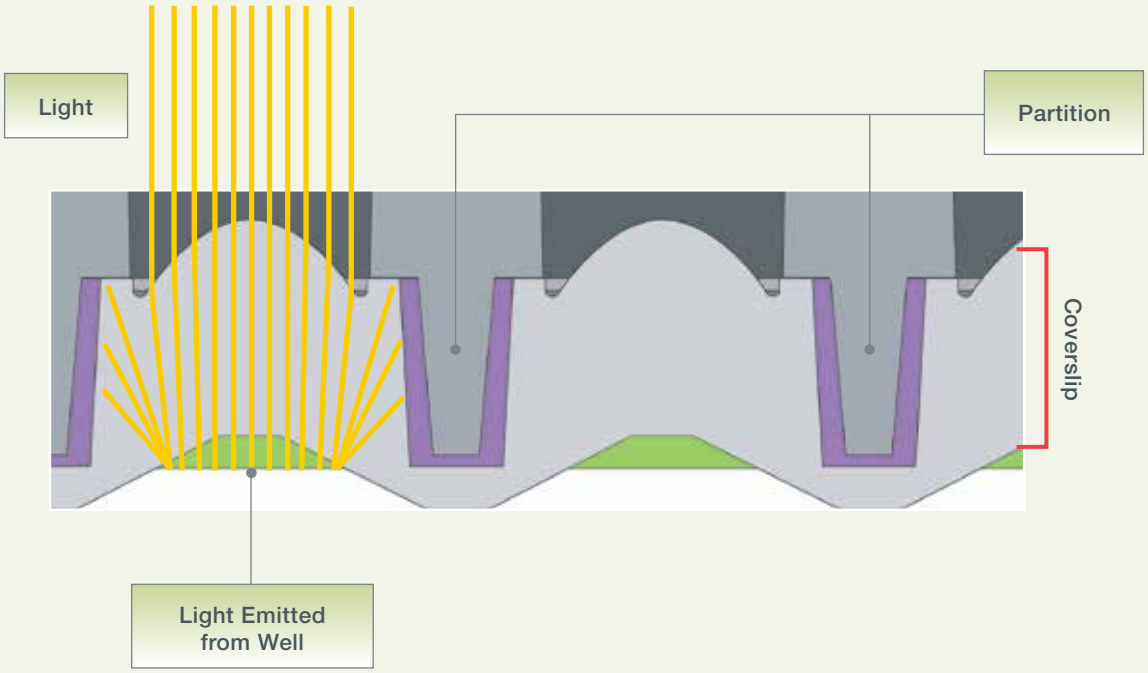

Fig. 4 | Cross-Section of the Partition and Coverslip for OPTIGEN

The allergen is one of the most critical components of an *in vitro* allergy test. HCD fully understands the need for tight control of both raw materials and extraction procedures in order to maintain consistent quality. **Figure 5** shows the process and approvals from raw material to extract.

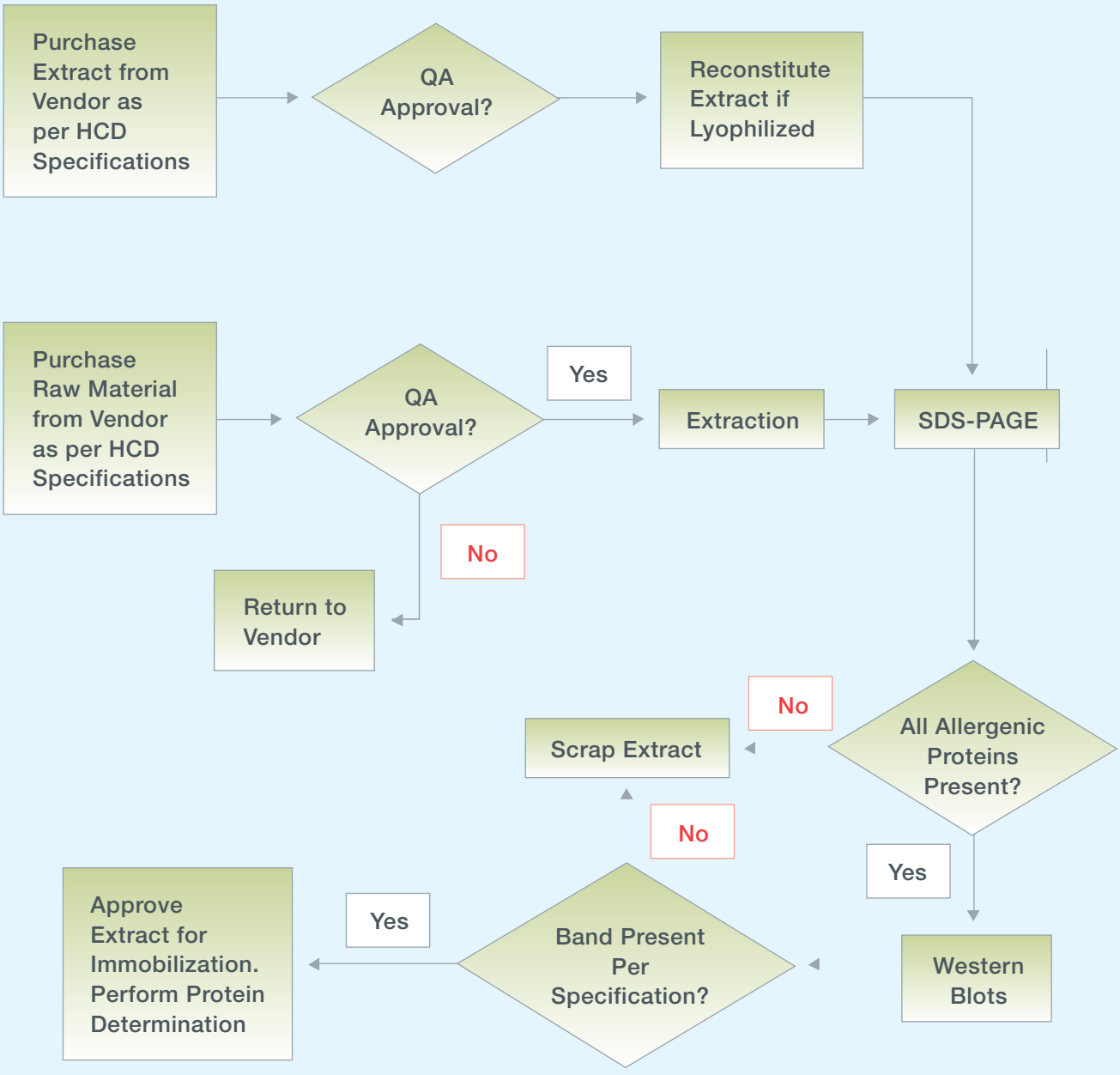

Fig. 5 | Process from Raw Material to Extract at HCD

Allergen vendors are screened and audited to ensure their ability to provide high quality and reproducible materials. Whenever possible, at least two sources of materials are identified for each allergen to ensure continuous supply. Extraction procedures are developed under Design Control guidelines and documented for production to allow for consistency and high quality. Each allergen is characterized and standardized to ensure that allergenic proteins are present and are immunologically active. Upon extraction, each allergen is analyzed by SDS-PAGE and major allergenic bands are identified. An example for Timothy grass can be seen in Figure 6. If the allergenic proteins are not present the extract is discarded and not used in product.

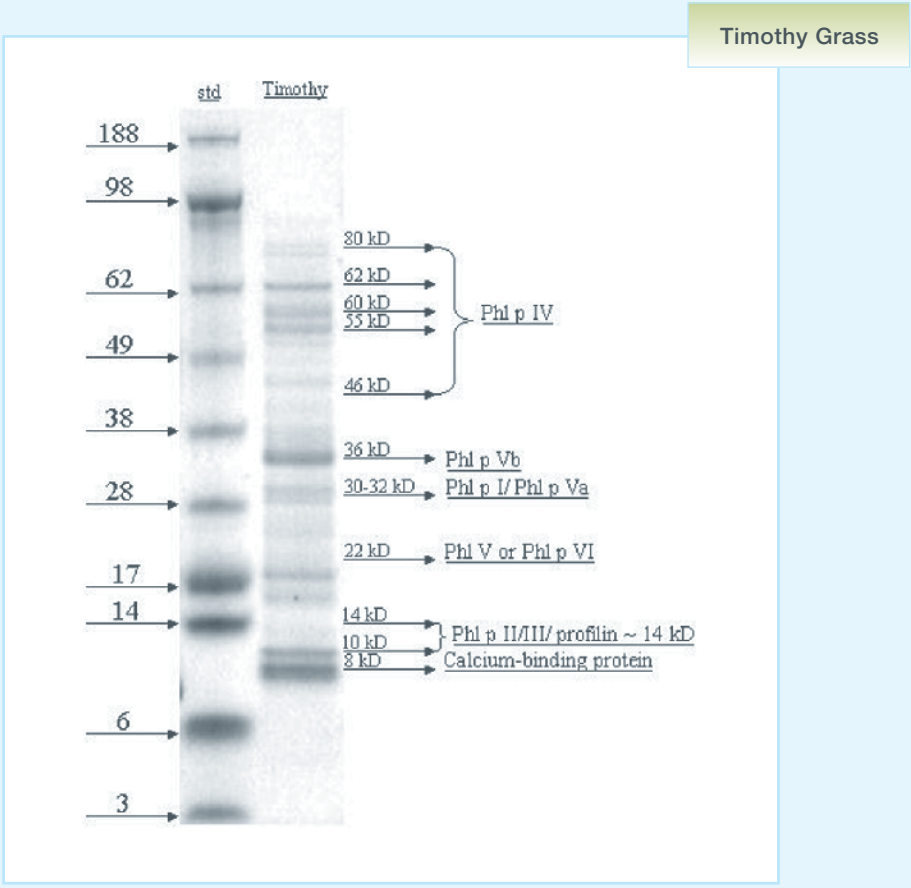

Fig. 6 | SDS-PAGE for Timothy Grass with Major Allergenic Proteins Identified

Immunoblots are performed on extracts that contain appropriate bands in the SDS-PAGE. HCD maintains a sera library with positive and negative serum for each extract. The positive sera are selected based on their response to different allergenic proteins in the extract. An example for Timothy grass can be seen in **Figure 7**.

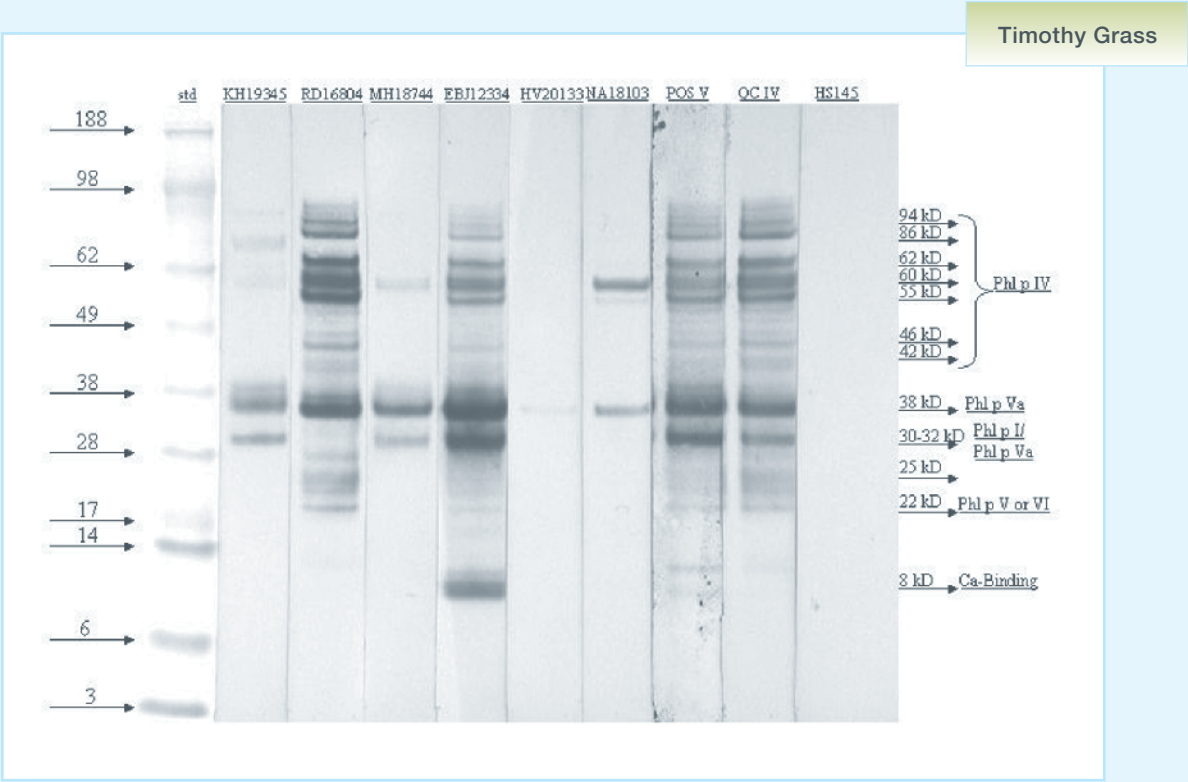

Fig. 7 | Immunoblots for Timothy Grass with Eight Positive and One Negative Serum Sample

Each allergen available with OPTIGEN has gone through the extensive characterization program described above. Allergenic proteins are identified and documented; a library of specific sera is available for testing in immunoblots and later in the assay for confirmation of performance. All the characterization and qualification information is documented per allergen and is available on the manufacturing floor.

IV Manufacturing Process

Extracts approved by the process described above are stored for use when needed. Each lot of allergen is tested at different concentrations for selection of the optimal immobilization concentration. The coverslips are treated through a proprietary method to enhance protein attachment. Immobilization of all allergens is done using specialized equipment that can accurately dispense in the nanoliter range **(Figure 8)**. The process is tightly controlled by determining the protein concentration of the extract prior to its application to the solid phase. Process control charts are produced to ensure that the extract concentration is within specification **(Figure 9)**. Trends can be seen over time and used to evaluate performance.

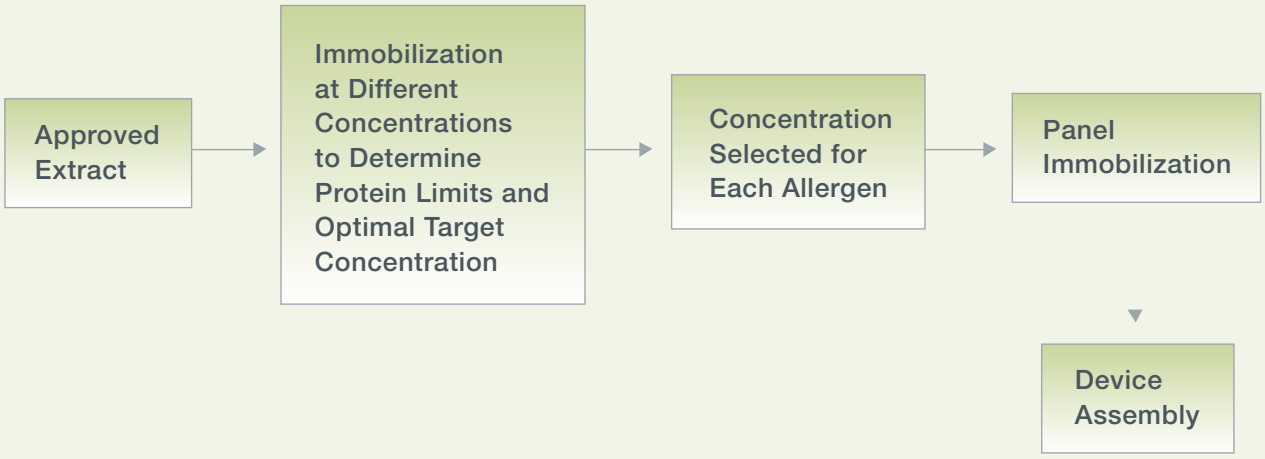

Fig. 8 | Summary of the Manufacturing Process for OPTIGEN

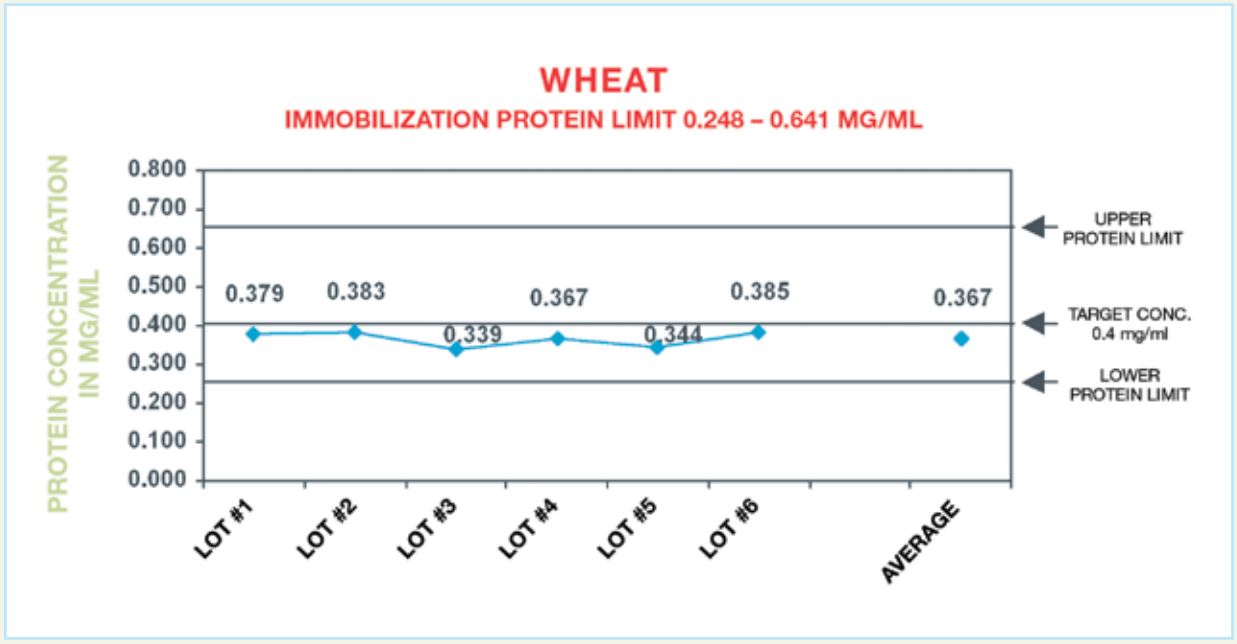

Fig. 9 | Process Control Chart for the Immobilization of Wheat over Six Production Lots

V Qualification Procedure

A random sample of assembled devices is tested with positive and negative control sera that have been qualified for OPTIGEN. The expected performance of these sera has been characterized for different allergens and is documented. The flow of the qualification procedure is shown in **Figure 10**.

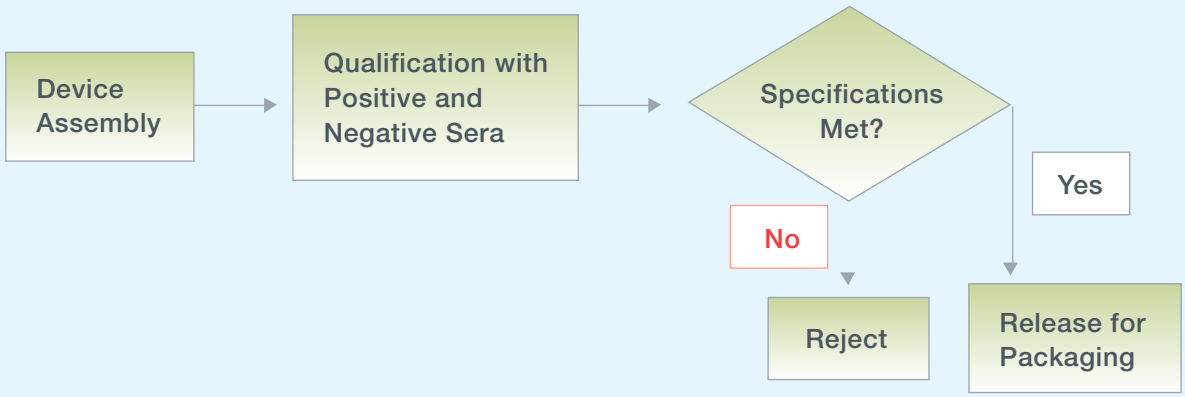

Fig. 10 | Summary of the Qualification Procedure for OPTIGEN

VI Universal Panel 20

The first marketed panel using OPTIGEN technology is the Universal Panel 20 **(Figure 11)**. This panel was selected with the aid of allergists and pediatricians in different countries and targets the most common allergens worldwide. This panel is ideal for pediatric use due to the low amount of serum sample needed for 20 results.

ALLERGEN	LATIN NAME
Birch, White	*Betula verrucosa (alba, pendula)*
Ragweed Mix I	*Ambrosia elatior/Ambrosia trifida*
Timothy Grass	*Phleum pratense*
Mugwort	*Artemisia vulgaris*
Latex	*Hevea brasiliensis*
Soybean	*Glycine max*
Rice	*Oryza sativa*
Codfish	*Gadus morhua*
Wheat	*Triticum aestirum*
Peanut	*Arachis hypogaea*
Egg white	*Gallus gallus*
Cow's Milk	*N/A*
Aspergillus	*Aspergillus fumigatus*
Cladosporium	*Cladosporium herbarum/Cladosporium cladosporiodes*
Alternaria	*Alternaria alternata (tenuis)*
Dog	*Canis*
Cat	*Felis domesticus*
Cockroach Mix	*Blatella germanica/Periplaneta americana*
Mite p	*Dermatophagoides pteronyssinus*
Mite f	*Dermatophagoides farinae*

Fig. 11 | Allergens in the Universal Panel 20

The performance of this panel has been evaluated in several studies. These include studies run at HCD with samples purchased from vendors, samples from Poland and Sweden, and two site evaluations—one done in France and one done in Germany. All evaluations were performed against Pharmacia CAP. **Figure 12** shows the overall assay performance with data collected at the evaluations in Germany and France. Independent operators not associated with HCD ran these evaluations after being trained by HCD personnel. The results show a sensitivity of 88%, a specificity of 94% an efficiency of 92% with a total of 1348 results for 20 allergens. 90% of the OPTIGEN results were within one class of Pharmacia CAP.

OPTIGEN

4	0	1	69	116	77
3	6	1	50	29	9
2	9	8	65	27	5
1	29	12	39	14	4
0	707	40	25	6	0
	0	1	2	3	4, 5 & 6

Pharmacia CAP

SENSITIVITY % 88%
SPECIFICITY % 94%
EFFICIENCY % 92%

TOTAL DATA POINTS: 1348

OPTIGEN

1	44	526
0	707	71
	0	1

Pharmacia CAP

OPTIGEN	vs.	CAP	
C	No.	%	W/1C
=	890	66.02%	90%
+1	203	15.06%	
-1	115	8.53%	
+2	79	5.86%	
-2	44	3.26%	
+3	7	0.52%	
-3	10	0.74%	
Total	1348	100.00%	

Fig. 12 | Performance of the Universal Panel 20 in Comparison to Pharmacia CAP.

Studies to determine the effect of high concentrations of total IgE were done by adding increasing concentrations of myeloma to a negative serum sample. The results showed no significant background increase with increasing concentrations of IgE up to 10,000 IU/ML.

Key Features of OPTIGEN:

- Approximately 300 µL of serum for 20 allergens.
- Assay time less than 5 hours: 2 hours serum incubation and 2 hours conjugate incubation.
- Semi-quantitative results reported in classes from 0 to 4.
- Availability of a positive control serum with suggested ranges for several allergens for quality control.
- Availability of a negative control serum for quality control.
- Minimal hands-on time.
- Easy set up, all allergens included in the device.
- No allergen inventory needed at the testing site.
- Easy procedure.
- Backwards compatible with CLA-1™ Luminometer instrumentation.

Hitachi Chemical Diagnostics, Inc.

With its headquarters in the heart of Silicon Valley and offices around the world, Hitachi Chemical Diagnostics is a global leader of *in vitro* allergy diagnostics with a legacy of innovation. Hitachi Chemical Diagnostics was the first company to introduce a multiple diagnostic test for allergies and one of the first to introduce a chemiluminescent assay system for the detection of IgE antibodies. In addition to OPTIGEN, HCD also produces the reliable CLA® Allergy Test and the AP 720S™ Semi-Automated Instrument.

Hitachi Chemical Diagnostics, an integral member of the Hitachi Group, works with industry leaders, laboratories and distributors around the world to provide the medical community access to the latest *in vitro* allergy testing technology. Hitachi Chemical Diagnostics is committed to innovation, heritage, and the strength of the Hitachi brand. Our products are marketed to over 40 countries worldwide. To learn more about Hitachi Chemical Diagnostics and OPTIGEN, please contact us or your local representative, or visit us on the web at **www.hcdiagnostics.com**.

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