CLINICAL EVALUATION

1. Purpose

To evaluate performance characteristic of House Dust Mite Test in a clinical routine, compared with a reference method, Immunocap HDM IgE Thermofisher Ref. 14410701 – 14410801 Lot. 126G/K118 batch N.16014 Exp: 2019/11.
The clinical laboratory involved in this study was “Centro Diagnostico Italiano - CDI” set in Milan (Italy). Mixed population investigated belongs to outpatients reporting to CDI with the request of House Dust Mite specific IgE detection.
The study was conducted from October 2018 to January 2019.
Official data report was filed in Folder - Project N. DPH016.

2. Statistical guidelines for assessing number of samples for a clinical trial study

In accordance with statistical guidelines, we have fixed an” interval of confidence” of 95%.
Our aim was to establish a prevalence estimation with a pre-determined precision level.
The dimension of the cohort to be investigated, can be calculated with the following formula:

$$N = \frac{Z_{crit^2} \cdot P_{exp} \cdot (1 - P_{exp})}{D^2}$$

where:

- **Z_{crit^2}** is the value of the “normal” in correspondence to fixed p-value = 1.96 for chosen level of confidence
- **P_{exp}** is the variance of the proportion $P = 0.1$ with regard to HDM allergic population incidence and considering the variance fixed in P=0.25 for PRIMA Lab Allergy total IgE test kit.
- **D^2**= 0.08^2 is the desired precision of the estimation.

Conclusion: N. 54 tests is the number obtained to get a significant statistical result.

3. Materials and Methods

PRIMA House Dust Mite Test Device pilot lot used: Lot 01P exp: 09/2020.
PRIMA House Dust Mite Test Diluent pilot lot used: Lot 01P exp: 08/2020.
Thermofisher Immunocap House Dust Mite IgE Ref. 14410701 – 14410801.
Unknown human sera were tested in parallel with PRIMA House Dust Mite Test and with Thermofisher Immunocap House Dust Mite IgE.
Samples with a concentration of House Dust Mite equal or higher than **0.35 IU/mL** were considered as positive (the average value, obtained with the reference method, expressed in concentration between *Dermatophagoides pteronyssinus* and *D.farinae* was considered) whilst samples with House Dust Mite average concentration lower than 0.35 IU/ml were considered negative (the input of the project DPH016 was to reach the cut-off value of 0.35 IU/mL).

Each sample was tested as indicated below, according to PRIMA House Dust Mite Test leaflet.

Component/step	Protocol
Sample volume	30 µl
Volume of diluent	2 drops
Reading time	5 minutes

Tab. 1: HDM Test protocol

4. Results

Device: Lot.01P exp: 09/2020
Diluent: Lot.01P exp: 08/2020

Nr.	ID Sample	PRIMA Lab HDM Test Device LOT01P Diluent LOT01P	Reference Method CDI phadia™ 250 Immunoassay Analyzers Thermo Fisher Scientific (Ua/mL)	Reference Method CDI phadia™ 250 Immunoassay Analyzers Thermo Fisher Scientific Average value (Ua/mL)
1	155007784602	Positive	D.f.: 2.69 D.p.: 2.48	2.585
2	158005803602	Negative	D.f.: 0.05 D.p.: 0.04	0.045
3	100044666602	Negative	D.f.: 0.03 D.p.: 0.03	0.03
4	157007996602	Positive	D.f.: 1.83 D.p.: 1.75	1.79
5	155007849602	Positive	D.f.: 58.1 D.p.: 32.8	45.45
6	105002286602	Negative	D.f.: 0.03 D.p.: 0.05	0.04
7	100046238602	Negative	D.f.: 0.03 D.p.: 0.03	0.03
8	106005316602	Negative	D.f.: 0.06 D.p.: 0.07	0.065
9	106005317602	Positive	D.f.: 2.28 D.p.: 1.10	1.69
10	144012281602	Positive	D.f.: 10.9 D.p.: 5.15	8.025
11	153002722602	Negative	D.f.: 0.07 D.p.: 0.08	0.075
12	146003718602	Negative	D.f.: 0.03 D.p.: 0.03	0.03
13	100047010602	Positive	D.f.: 7.93 D.p.: 12.6	10.265
14	107003450602	Negative	D.f.: 0.18 D.p.: 0.18	0.18

15	100052950602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
16	157009508602	Positive	D.f.: > 100 D.p.: > 100	>100
17	144014121602	Negative	D.f.: 0.10 D.p.: 0.09	0.095
18	156003021602	Positive	D.f.: 3.12 D.p.: 4.50	3.81
19	1000532068602	Positive	D.f.: 0.63 D.p.: 0.67	0.65
20	163010512602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
21	141003053602	Negative	D.f.: 0.08 D.p.: 0.07	0.075
22	154003343602	Positive	D.f.: 65.1 D.p.: 98.8	81.95
23	163011685602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
24	100059869602	Negative	D.f.: 0.02 D.p.: 0.03	0.025
25	146004892602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
26	150010566602	Positive	D.f.: 89.6 D.p.: 60.3	74.95
27	100061435602	Negative	D.f.: 0.04 D.p.: 0.13	0.085
28	100061786602	Negative	D.f.: 0.00 D.p.: 0.03	0.015
29	106006746602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
30	163012417602	Negative	D.f.: 0.02 D.p.: 0.02	0.02
31	100063284602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
32	100063418602	Negative	D.f.: 0.00 D.p.: 0.01	0.005
33	101015678602	Positive	D.f.: 22.4 D.p.: 14.9	18.65
34	105003364602	Negative	D.f.: 0.00 D.p.: 0.00	0.00
35	153003841602	Positive	D.f.: 1.90 D.p.: 2.09	1.995
36	163012828602	Positive	D.f.: 29.0 D.p.: 54.1	41.55
37	155007784600	Positive	D.f.: 2.78 D.p.: 2.65	2.715
38	157008456600	Positive	D.f.: 48.10 D.p.: 35.30	41.7

39	151006523600	Positive	D.f.: 83,80 D.p.: nn	83.8
40	155010243600	Positive	D.f.: 1.39 D.p.: 1.52	1.455
41	149004838600	Negative	D.f.: 0.42 D.p.: 0.40	0.41
42	101013655600	Positive	D.f.: >100.00 D.p.: 41.90	70.95
43	163011142600	Negative	D.f.: 0.40 D.p.: 0.11	0.255
44	160007029600	Positive	D.f.: 1.23 D.p.: 0.67	0.95
45	142001265600	Negative	D.f.: 0.22 D.p.: 0.18	0.2
46	100052671600	Negative	D.f.: 0.22 D.p.: 0.21	0.215
47	100053629600	Negative	D.f.: 3.66 D.p.: 0.26	1.96
48	144014498600	Positive	D.f.: 1.87 D.p.: 2.04	1.955
49	144014539600	Positive	D.f.: 0.79 D.p.: 0.80	0.795
50	155009505600	Positive	D.f.: 3.25 D.p.: 5.93	4.59
51	144014611600	Positive	D.f.: 0.54 D.p.: 0.60	0.57
52	157009949600	Positive	D.f.: 2.30 D.p.: 2.55	2.425
53	158007281600	Negative	D.f.: 0.15 D.p.: 0.18	0.165
54	159008669600	Positive	D.f.: 0.27 D.p.: 0.28	0.275

Tab. 2: Results table

Samples assayed: 54

	Immunocap HDM Ig E Thermofisher	
HDM Test	**Positive**	**Negative**
Positive	25	1
Negative	2	26

Tab. 3: Results summary table

Specificity = (26 /26 + 1) * 100 = **96.29%** (CI 95%: 89.17 – 100%)
Confidence interval was calculated with the following formula:
spe ± 1,96√spe(1-spe)/tot number of negative

Sensitivity = (25/25+2) * 100 = **92.59%** (CI 95%: 82.71 – 100%)
Confidence interval was calculated with the following formula:
se ± 1,96√se(1-se)/tot number of positive

Accuracy = (25+26/25+26+1+2) * 100 = **94.4%** (CI 95%:88.34 – 100%)
Confidence interval was calculated with the following formula:
acc ± 1,96√acc(1-acc)/tot number of samples

5. Conclusion

In accordance with data reported, we claim a specificity value of 96.29%.
On the contrary, the sensitivity value claimed is defined in 92.59%,
Accuracy, also named Overall Agreement is settled in 94.40%.

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