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## Hypersensitivity pneumonitis: A new strategy for serodiagnosis and environmental surveys

Anne-Pauline Bellanger, Gabriel Reboux, Adeline Rouzet, Coralie Barrera, Steffi Rocchi, Emeline Scherer, Laurence Millon

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1 **Hypersensitivity pneumonitis: a new strategy for serodiagnosis and environmental**  
2 **surveys**

3 Anne-Pauline Bellanger PharmD PhD, Gabriel Reboux PhD HDR\*, Adeline Rouzet PhD,  
4 Coralie Barrera PhD, Steffi Rocchi PhD, Emeline Scherer Pharm D PhD, Laurence Millon  
5 Professor

6  
7 Department of Parasitology Mycology, University Hospital of Besançon, UMR/CNRS 6249  
8 Chrono-Environnement research team, University of Bourgogne- Franche-Comté, France

9  
10 \*Corresponding author: Gabriel Reboux Jean Minjoz University Hospital, 2 Bd Fleming  
11 25030 Besançon Cedex France Tel +33 3 70 63 23 56 e-mail: [gabriel.reboux@univ-fcomte.fr](mailto:gabriel.reboux@univ-fcomte.fr)

12  
13 Bellanger A-P, ORCID: 0000-0003-4144-476X  
14 Reboux G, ORCID: 0000-0002-7923-0858  
15 Rouzet A, ORCID: 0000-0002-9327-2165  
16 Barrera C, ORCID: 0000-0002-3568-8007  
17 Rocchi S, ORCID: 0000-0001-9262-1127  
18 Scherer E, ORCID: 0000-0003-2123-3703  
19 Millon L, ORCID: 0000-0001-5939-3409

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23 **Abstract (148 words)**

24 We propose a strategy for serodiagnosis of hypersensitivity pneumonitis (HP): 1) question  
25 patients about their private or occupational activity, or visit him on site; 2) select panels of six  
26 somatic specific antigens appropriate for each type of exposure; 3) and use ELISA to test  
27 concomitantly two recombinant antigens highly specific to Farmer's lung, Metalworking-fluid  
28 HP, and for Bird fancier's lung. The serodiagnosis provides an immunological argument that  
29 may complete radiological, functional lung exploration and clinical features; 4) If the  
30 serodiagnosis is negative but the suspicion of HP is strong, a microbial analysis of the  
31 patient's specific exposure is conducted; 5) "A la carte" antigens are produced from the  
32 microorganisms isolated in the patient's environment sample and tested; 6) Finally, the patient  
33 may be asked to undergo a specific inhalation challenge with the offending antigens in a  
34 safety cabin, or to avoid his usual environment for a few days.

35

36 **Key words**

37 hypersensitivity pneumonitis; serodiagnosis; recombinant antigen; etiologic agents;  
38 environmental survey

39

40 **Highlights**

41 A six-step strategy for serodiagnosis of hypersensitivity pneumonitis is recommended.

42 Three kinds of antigens must be used: somatic, recombinant and "a la carte" antigens.

43 Microbiological surveys are helpful to select antigens or make "a la carte" antigens.

44 As proof, re-exposure to the natural environment or commercial extract is necessary.

45

46 **Introduction (3136 words)**

47 Hypersensitivity pneumonitis (HP) is a group of inflammatory interstitial lung diseases caused  
48 by repeated inhalation of antigenic substances from a large variety of agents over several  
49 years. In most cases, repeated contact results in sensitization: no *in situ* growth seems  
50 required. From a clinical point of view, HP diagnosis is difficult and based on a combination  
51 of clinical, radiological, functional, cytological, histological and biological criteria [1,2]. The  
52 current classification of HP, that differentiates acute, subacute and chronic forms, is accepted  
53 worldwide [3]: in the acute form, flu-like symptoms predominate and include fever, cough,  
54 myalgia and arthralgia, which occur 4 to 8 h after exposure. Dyspnea is not constant. The  
55 subacute form appears gradually and associates cough and dyspnea, with sometimes severe  
56 respiratory failure. The chronic form includes coughing, dyspnea, fatigue and weight loss [4].

57 Recently, the classic HP classification has been under debate, essentially because this  
58 classification does not take into account the role of the mode of exposure and the evolution of  
59 the disease. The prognosis and evolution of HP seem to depend on the type and pattern of  
60 exposure. A new classification with two clusters has been suggested: in type 1, massive and  
61 intermittent exposure, as in FLD, may lead to emphysema with chronic airflow obstruction  
62 and, in type 2, chronic exposure to a low level, as in bird fanciers, may lead to fibrosis with a  
63 restrictive pattern. Vasakova and coll. proposed two forms: acute HP /inflammatory (form A)  
64 and Chronic HP/fibrotic HP (form B) [4-6].”

65

66 First, serological analyses are mainly used to rule out the diagnosis of HP in favor of other  
67 respiratory pathologies. A survey of 107 patient files for which a request for PHS serology  
68 had been made (including positive and negative results) indicates that the final diagnosis of  
69 respiratory pathology was different from HP for 76.6% of patients. BAL was only performed

70 in 40% of cases. The final diagnoses were: idiopathic pulmonary fibrosis (IPF), nonspecific  
71 interstitial pneumonia (NSIP), diffuse interstitial pneumonia (DIP), chronic obstructive  
72 pulmonary disease (COPD), sarcoidosis, pulmonary infection, and asthma. (Unpublished  
73 personal data). Secondly, when HP diagnostic is established, serology helps to identify the  
74 etiology of HP. The serodiagnosis will help to determine the avoidance measures to  
75 implement [7].

76 Since the 1960s [3], several new environmental contexts of antigen exposure associated with  
77 HP have been reported [6,9].

78 Bird fancier's lung is the most prevalent form of HP worldwide, accounting for 66-68% of all  
79 forms of HP in three independent studies [10-12]. The estimate for the prevalence of BFL  
80 ranges from 6 to 20% of exposed pigeon breeders [13-14] and from 0.5% to 7.5% for  
81 budgerigar's lung [15].

82 This disease is induced by exposure to avian proteins present in the droppings, sera, feathers  
83 and bloom of a variety of birds [16]. BFL has been reported primarily after exposure to  
84 pigeons, doves, canaries, poultry and birds from the Psittaciforme order [14,17], but also after  
85 exposure to wild birds such as owls or geese [18].

86 Farmer's Lung Disease (FLD) is described with a large variety of identified antigens  
87 including actinomycetes (*Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*) and  
88 molds (*Aspergillus*, *Absidia*, *Penicillium*, *Wallemia*, *Fusarium*) [8,19-22]. New FLD etiologic  
89 agents have been identified along with changes in professional practices [23,24].

90 In the 1990s, another form of HP emerged called Metalworking-fluid hypersensitivity  
91 pneumonitis (MWF-HP). It is prevalent among machinists of the automobile industry who are  
92 exposed to metalworking fluids (MWF) contaminated by *Mycobacterium immunogenum* (MI)  
93 [25,26]. Wood working is also an important professional circumstance for HP risk [27,28].

94 Domestic HP and hobbies related HP to common microorganisms have been increasingly  
95 reported over the past few years. More than 30 specific molds and mycobacteria have been  
96 identified as playing a causative role [29]. Among the many factors and circumstances that  
97 contribute to the development of domestic HP are contaminated humidifiers, faulty ventilation  
98 systems and jacuzzis (*Mycobacterium avium*) [30,31]. Even HP due to leisure activities [32-  
99 34] are now recognized [14].

100 A query on <http://www.ncbi.nlm.nih.gov/> with the following keywords: hypersensitivity  
101 pneumonitis (HP), bird fanciers Lung, pigeon breeder's disease, farmer's lung and other  
102 specific disease names derived from occupational activities followed by each etiology with  
103 their synonyms, evaluated the number of publications related to the different etiologies of HP.  
104 Thus, by families of similar etiologies we obtained the following number of publications (in-  
105 brackets) for: *Saccharopolyspora rectivirgula* (= *Micropolyspora faeni*) (632), other  
106 actinomyces (148), avian proteins (627), *Aspergillus* (348), *Penicillium* (33), yeasts (32),  
107 mucorales (30), others fungi (65), mycobacteria (72), enterobacteria (16), eatable fungi (20),  
108 mites (33), chemical product (68), biological product (30) [35].

109 Complex cases of several concomitant types of exposure are often seen in routine practice.

110 A farmer may breed exotic birds and present positive serological reactions to both birds and  
111 FLD antigens, and even to mite antigens (*Acarus siro*) if he is producing cheese [36]. Another  
112 demonstration of the importance of using the relevant specific antigen to obtain useful  
113 serology results was shown in diagnosing sausage worker lung with *Penicillium nalgiovense*.  
114 Exposure to several *Penicillium* species is very common. However, sausage workers  
115 specifically react to *P. nalgiovense* although they are concomitantly exposed to yeasts  
116 and molds in their workplace. Similarly, cheese workers specifically react to antigens from  
117 *Acarus siro* while they are exposed to domestic mites. Sometimes a patient is exposed without

118 being aware of it (i.e goose droppings in a chimney) or because of a common everyday object  
119 (i.e down pillows). [ 37,38]

120 The antigen panel to test may thus be selected based on the professional and domestic context  
121 of the patient [39]. This is why, more and more often, an environmental survey of the  
122 patient's exposure site is required to obtain samples that will be used to choose or produce  
123 specific antigens [36,40]. Improved species identification can be obtained thanks to  
124 molecular biology, progressively extending the list of new etiologic agents, since specific  
125 antibodies of the etiologic agent are present in HP patient serum [41,42]. Serodiagnosis  
126 techniques are being improved, especially with the development of more standardized  
127 recombinant antigens (RAg) [43-46]. Moreover, some RAg are shared by species involved in  
128 different HP, and one of the common peptide epitopes was shown to be able to differentiate  
129 patients from healthy exposed farmers [47].

130 We have synthesized our experience of HP serodiagnosis and environmental surveys and now  
131 propose a new strategy for routine serodiagnosis. The present strategy is based on our  
132 experience for the choice of a limited number of somatic antigens for each panel for each HP  
133 circumstances [39] and on a selection of RAg for the common antigenic determinants between  
134 the different HP [47].

135

### 136 **Strategy for HP immunological diagnosis**

137 Serodiagnosis of HP in our lab in Eastern France is based on 6 steps:

- 138 1) questioning the patient about his private or occupational activity, or visiting him on  
139 site
- 140 2) conducting precipitin research with several standardized panels (Table 1)
- 141 3) using ELISA level of recombinant antigens for FLD, BFL and MWF-HP

- 142 4) examining the patient's environment by culture or QPCR measurement  
143 5) producing and testing "a la carte" antigens from the environment  
144 6) suggesting to the patient an exposure challenge in his usual domestic or professional  
145 environment

146

#### 147 **Questioning patient about his private or occupational activity, or visiting him on site**

148 The initial step of the sampling strategy is a detailed questionnaire including the habits and  
149 tasks of the patient [6] but also of the other people present in the same environment.  
150 Observation on the sampling site is always better than asking the patient to do the sampling  
151 himself. The more accurate and precise the sampling is, the more useful it will be to further  
152 advise the patient regarding prevention and eviction measures.

153 The identification of the etiological agent is crucial for the diagnosis, preventive measures and  
154 prognosis of the disease. The relationship between exposure and disease may be relatively  
155 obvious in occupational HP exposed to a limited variety of antigens (i.e. MWF-HP, BFL), but  
156 in domestic HP cases it is often unclear, making it difficult to identify the antigen. The  
157 etiological agent fails to be identified in up to 60% of HP cases [48-50]. Special cases do  
158 exist and justify an individual investigation (questioning, visit on site, sampling and  
159 microbiological analysis).

160

#### 161 **Conducting precipitin research with several standardized panels**

162 Antigens used are either commercial antigens or « homemade » antigens. Four of the seven  
163 antigens tested in the study by Fenoglio and Coll. differentiated patients from controls with a  
164 predictive positive value of around 73%, but required the use of specific antigens in eight of  
165 11 false negative results (n= 122 including 31 cases of HP) [39]. Patients living in tropical



166 and arid climatic zones should benefit from tests taking into account the species present in  
167 these specific areas. However, the choice of antigen panel by profession allows the  
168 identification of the causes of HP in most cases. For FLD or domestic lung we use 2 panels of  
169 6 antigens composed of fungal or actinomycetes antigens. The antigens in the second panel  
170 are less frequently responsible compared to those in panel 1. (Table1). Their nature, quality  
171 and standardization may thus vary and impact serology results. Different types of antigens  
172 exist with different characteristics.

173 Total extract antigens are prepared from the phenol extraction of material manipulated by  
174 patients. This type of antigen is a variable mixture of antigens from bacteria, mold and mites.  
175 These antigens are appropriate for a first screening of global sensitization [51,52].

176 Somatic antigens are obtained from the micro-organisms present in the material manipulated  
177 by patients. Somatic antigens are made up of a mosaic of antigenic fractions and are more  
178 standardized than total antigens.

179 Proteic purified antigens are obtained after enzymatic lysis of cell wall polysids, protein  
180 acidic precipitation and acetone purification.

181

182 Precipitation reactions are semi-quantitative (double diffusion (Ouchterlony technique),  
183 electrosyneresis, immuno-electrophoresis) and allow a macroscopic visualization of the  
184 antigen-antibody reaction by generating precipitation arcs [53-55].

185 For the Western blotting (WB) technique, antigenic proteins are first separated by  
186 electrophoresis then transferred onto a nitrocellulosis membrane and revealed with an enzyme  
187 labeled anti-globulin. WB performed with patient sera provides complex profiles, difficult to  
188 differentiate from those of exposed controls [56]. This is due to the high number of proteins in

189 somatic antigens from micro-organisms and makes it difficult to select reproducible specific  
190 protein as a marker of the disease [57].

191

192 **Using ELISA level of recombinant antigens for FLD, BFL and MWF-HP**Recombinant  
193 antigens are the most recent ones [43-46,58]. They are obtained after cloning a specific gene  
194 coding for a unique antigenic protein. The DNA sequence is inserted into a plasmid. After  
195 transforming bacteria or yeast with this plasmid, a large quantity of the specific protein is  
196 produced. This is the most standardized way to produce antigens. However, these antigens  
197 correspond to a single protein and will thus reflect a limited part of the complex immunologic  
198 signals of the disease [43-46,58]. Numerous RAg were created, but only some of them were  
199 selected for three HP serodiagnoses: MWF-HP (Acyl-CoA-dehydrogenase (Acyl-CoA) and  
200 dihydrolipoyl dehydrogenase (DHDH)), FLD (SR17 hydroperoxidase (SR17) &  
201 Dihydrolipoamide dehydrogenase (DLDH) and BFL (Immunoglobulin lamda-  
202 likepolypeptide-1 (IGLL1) and proproteinase E (ProE) [43-46]. They can be used for ELISA  
203 (Enzyme Linked ImmunoSorbent Assay) with sensitivity and specificity reaching 80% and  
204 90%, respectively, for MWF-HP [45], 83% and 77% for FLD [44] and 76% and 100% for  
205 BFL [46]. The rate may be even higher if a combination of several antigens is used.

206

207 ELISA (Enzyme Linked ImmunoSorbent Assay) includes all the immunological assays with  
208 enzymatic labeling and antigen immobilization to a solid surface. This technique presents  
209 several advantages. It is quantitative, fast, and requires only a low volume of serum. The  
210 technique may be performed automatically, and several antigens may be coated onto the  
211 microtiter plate, including recombinant antigens [55]. The main drawback of ELISA is the  
212 high intra assay variability, which is around 10-20%.

213 Specific immunoglobulin antibodies of the etiologic agent are present in HP patient serum.  
214 The most frequently used techniques are immuno-precipitation methods (double diffusion,  
215 electrosyneresis, immunoelectrophoresis) and immuno-assays (ELISA). Great variations of  
216 sensitivity and specificity are due to many factors, from the criteria used to diagnose HP to the  
217 diversity of immunological techniques available [59]. The technique of electrophoresis that is  
218 using six antigens, extracted from micro-organisms isolated in contaminated hay, allowed us  
219 to diagnose FLD patients with a positive predictive value of 73% and a negative predictive  
220 value of 85% [39]. Similarly for BFL diagnosis, it was shown that serological arguments were  
221 in accordance with diagnosis in 87% of cases using antigens from the patient's birds versus  
222 20% using a commercial antigenic panel or "homemade" antigens [60,61]. A recent  
223 comparison of dropping extracts from three bird species showed cross reactions between bird  
224 species and some highly specific antigens [63]. For rare bird species, using total extract from  
225 the bird's feces is often the most efficient way to diagnose BFL [60]. The crucial factor  
226 influencing the performance of serology methods is to test the accurate offending antigens.  
227 Humoral response may be modulated by different circumstances. Avoidance of the offending  
228 antigen modifies serology results significantly [40,64]. It is estimated that around 5 years are  
229 needed to observe a 50% decrease in the number of precipitin arcs. This was observed in a  
230 population of farmers who had left their farms upon retirement [65]. Smoking and  
231 immunosuppression significantly decrease humoral response [2,66]. Treatments with  
232 glucocorticosteroids do not modify serology results significantly (unpublished data).  
233 As a rule, a microorganism is considered as an etiologic agent of HP when a link is  
234 established between the microbiologic environment of the patient and his immune response.  
235 In some cases, exposed subjects develop antibodies to HP- recognized agents but never  
236 develop the disease [1]. In practice, to provide proof of the type of exposure with an  
237 indication of the responsible agent, an inhalation challenge in safe conditions may be

238 performed, or alternatively, a “natural avoidance test”, where the suspected antigen is  
239 withdrawn from the patient’s environment [40,67]. In most cases, the recognized HP etiologic  
240 agent is the most discriminant in serological tests to distinguish patients from asymptomatic  
241 controls. Double diffusion (Ouchterlony) is often considered as not being correlated with HP  
242 [68]. Electrosyneresis (ES) is slightly more sensitive than double diffusion with a number of  
243 arcs deviating from 0 to 15. This is the reason why 2 to 5 arcs are necessary to consider the  
244 reaction positive. The diagnostic performance of ELISA and precipitin assays was assessed by  
245 analyzing the Receiver Operating Characteristics (ROC) curve. The threshold that best  
246 distinguished patients from controls was determined with the most effective combination of  
247 sensitivity and specificity for each serological technique [59].

248

249 **Examining the patient’s environment by culture or QPCR measurement** Measuring  
250 micro-organisms present in the workplace by air impaction is possible in environments  
251 showing little contamination [70]. In case of highly contaminated areas leading to saturation  
252 of culture media, air sampling may be performed using air pumping on a filter, sponge or  
253 liquid [71,72]. Substrate samples (hay, working fluids) should be analyzed by culture [73] or  
254 QPCR methods [74]. Hay batches are first stored at  $-20^{\circ}\text{C}$  to kill mites which bias mold  
255 quantification. The results should be interpreted according to their weight (quantitative  
256 analysis). An electrostatic dust collector (EDC) exposed 4 to 10 weeks or dust vacuum  
257 sampling on 1 to  $2\text{m}^2$  of floor should be used in indoor sampling [75,76]. Surface sampling by  
258 swabbing gives qualitative results because it is only representative of a limited sampled area.  
259 Multiplying types of culture media and incubation temperatures is the most effective way to  
260 isolate a large number of microorganisms. Culture media containing dichloran reduces the  
261 growth of some fungi and prevents the growth of Mucorales, which are very extensive  
262 species. However, cultivation methods provide imperfect quantification of major viable

263 species (due to the dilution effect of the sample obtained after several hours of pumping)  
264 while QPCR allows the quantification of both viable and non-viable species including species  
265 in low concentrations [77]. It is important to note that QPCR quantify only the target species  
266 depending on an a priori choice. Molecular biology using DNA sequencing has improved the  
267 reliability of species identification. For example, these techniques have allowed us to confirm  
268 the presence of *Penicillium nalgiovense* in an industrial sausage factory, and that of *Acarus*  
269 *siro* in a Comte - or Mimolette cheese factory [36]. Applying these current techniques saves  
270 time and improves reliability in identifying new HP etiologic agents such as *Mycobacterium*  
271 *immunogenum* in metalworking fluid HP [74]. Recently, DNA metabarcoding analysis was  
272 able to extend the list of indoor fungi sampled by EDC (viable, unviable or unknown species)  
273 [42].

274

#### 275 **Producing and testing “a la carte” antigens from the environment**

276 No commercial kit currently exists for this approach which is possible only in highly specialized centers  
277 at present. The "case-by-case" study of environmental situations is feasible for all laboratories  
278 but requires dedicating an appropriate number of trained technicians to sampling,  
279 microbiological analysis, customized antigen production and serology. These skills exist in  
280 several countries (e. g. Finnish Institute of Occupational Health (Kuopio/ Finland), National  
281 Jewish Medical and Research Center (New York/USA)). Other centers suggested testing  
282 panels combining several antigens (e.g. ARUP labs (Salt Lake City/ Utah, Allergy  
283 immunology Diagnostic Lab center, Milwaukee, Wi). However, the availability of a  
284 commercial kit (using recombinant antigens) would facilitate the generalization of HP  
285 serodiagnosis around the world.

286 Based on our experience, the complete analysis for a MWF lung patient or BFL would cost 40  
287 to 50 € for microbiology, 30 to 60 € for serology and 150 € for the production of a new  
288 antigen. On the other hand, for more complex environments (farms, housing,...) the costs may  
289 vary depending on whether we perform the analysis with a standard panel of 6 antigens (90 €)  
290 or whether we do a "a la carte" approach with the microbiological analysis costing between  
291 650 and 900 €.

292

293 **Suggesting to the patient an exposure challenge in his usual domestic or professional**  
294 **environment**

295 Specific inhalation challenge tests (SICs) aimed to confirm the causal link between the  
296 suspected antigen and HP. A purpose re-exposure of the subject to the suspected agent in his  
297 natural environment or to commercially available extracts may be proposed [6,9,78]. These  
298 SICs require close clinical monitoring when they are performed [79]. The test is considered  
299 positive if there is a decrease in forced vital capacity (FVC) of more than 15% and lung  
300 diffusing capacity of carbon monoxide (DLCO) of more than 20% or a recurrence of clinical  
301 symptoms (desaturation, hyperthermia, cough, dyspnea) and radiological signs (infiltrates)  
302 [6].

303

304 **Conclusions**

305 Individuals with HP develop this disease in a number of different environmental contexts and  
306 are thus exposed to different etiologic agents present in these contexts.. Selecting the relevant  
307 antigen for serology testing is a key step, definitely influencing the performance of  
308 serodiagnosis. In addition to improving the standardization of antigen testing quality,

309 recombinant antigens should lead to a much better discrimination between patients and  
310 healthy exposed individuals, with optimized specificity / sensitivity, therefore making it  
311 possible to avoid invasive examinations.

312

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318

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320 The authors have no conflicting interests.

321

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507

508 Table 1: Total extract, somatic and recombinant antigens for precipitins/antibodies research  
 509 for each environmental circumstance/setting  
 510

Circumstance	<i>Total extract antigens</i>		
<b>Bird breeder</b>	(Standard panel) dropping for:	pigeon, parakeet, chicken, duck, canary,	
	dropping for rare species	bengali, chardonneret, quail, beijing duck, dove, peru dove, sydney diamond, turkey, pheasant, hawk (pilgrim and gerfaut), owl, swallow, inseparable, green kakariki, Mandarin, goose, parrot (amazon and gray of Gabon), parakeet calopsitte, Padda, peacock, serin	
<b>Wood worker</b>	sawdust, fir, poplar, niagon, iroko, sapeli		
<b>Miller &amp; baker</b>	corn, wheat, oats, rye, barley		
<b>Pets shop worker</b>	Fecal extract of rodents	bat, guinea pig, hamster, ind pig, gerbil, white mouse	
Circumstance	<i>Somatic antigens</i>		
<b>Farmer (panel 1)</b>	<i>Lichtheimia corymbifera</i>	<i>Walleimia sebi</i>	<i>Eurotium amstelodami</i>
	<i>Saccharopolyspora rectivirgula</i>	<i>Thermoactinomyces vulgaris</i>	<i>Saccharomonospora viridis</i>
<b>Farmer (panel 2)</b>	<i>Fusarium solani</i>	<i>Aspergillus versicolor</i>	<i>Aspergillus ochraceus</i>
	<i>Penicillium chrysogenum</i>	<i>Rhodotorula rubra</i>	mesophilic <i>Streptomyces</i>
<b>Housing (panel 1)</b>	<i>A. versicolor</i>	<i>P. chrysogenum</i>	<i>Cladosporium sphaerospermum</i>
	<i>Stachybotrys chartarum</i>	<i>Mucor racemosus</i>	<i>Alternaria alternata</i>
<b>Housing (panel 2)</b>	<i>Schizophylum commune</i>	<i>Trichoderma pseudokoningii</i>	<i>Fusarium oxysporum</i>
	<i>Acremonium strictum</i>	<i>Rhodotorula rubra</i>	<i>Laceyella sacchari</i>
<b>Cheese-maker</b>	<i>Acarus siro</i>	<i>Penicillium camemberti</i>	<i>Penicillium roqueforti</i>
	<i>Mucor spinolosum</i>	<i>Geotrichum candidum</i>	
<b>Pork-butcher</b>	<i>Penicillium nalgiovense</i>	<i>G. candidum</i>	
<b>Jacuzzi</b>	<i>Mycobacterium avium</i>	<i>Mycobacterium phocaicum</i>	
<b>Metalworking fluid</b>	<i>Mycobacterium immunogenum</i>	<i>Bacillus simplex</i>	<i>Pseudomonas oleovorans</i>
		<i>Fusarium solani</i>	
<b>Plaster-maker</b>	<i>S. rectivirgula</i>	<i>T. vulgaris</i>	<i>Aspergillus fumigatus</i>
	<i>Penicillium frequentans</i>	<i>P. chrysogenum</i>	<i>A. versicolor</i>
<b>Sugar cane</b>	<i>L. sacchari</i>	<i>S. viridis</i>	<i>Thermocrisum municipale</i>
	<i>T. vulgaris</i>	<i>A. fumigatus</i>	
<b>Compost</b>	<i>S. rectivirgula</i>	<i>T. vulgaris</i>	<i>S. viridis</i>



	<i>A. fumigatus</i>	<i>Thermobifida fusca</i>	mesophilic <i>Streptomyces</i>
<b>Wood worker</b>	<i>P. chrysogenum</i>	<i>P. frequentans</i>	
<b>Fungi maker</b>	<i>S. viridis</i>	<i>T. vulgaris</i>	
<b>Wine producer</b>	<i>Botrytis cinerea</i>	<i>P. chrysogenum</i>	<i>Saccharomyces cerevisiae</i>
	<i>Mucor racemosus</i>	<i>A. versicolor</i>	
<b>Wind instrument player</b>	<i>R. rubra</i>	<i>P. chrysogenum</i>	<i>Phoma glomerata</i>
	<i>E. amstelodami</i>	<i>F. oxysporum</i>	
<b>Humidifier</b>	<i>C. sphaerospermum</i>	<i>A. strictum</i>	<i>Exophiala dermatitidis</i>
<b>Recombinant antigens</b>			
<b>Bird breeder</b>	Immunoglobulin Lamda-Like polypeptide-1 (IGLL1)		Proproteinase E (ProE)
<b>Farmer</b>	SR17 Hydroperoxidase (SR17)		Dihydrolipoamide Dehydrogenase (DLDH)
<b>Metalworking-fluid worker</b>	Acyl-CoA-Dehydrogenase (Acyl-CoA)		Dihydrolipoyl Dehydrogenase (DHDH)

511

512 Note: The antigens in the second panel are less frequently responsible compared to those in

513 panel 1.

# An environmental and serological strategy for the diagnosis of HP



1. Kind of exposure  
(birds, occupational, dwelling ?)

**Serological analysis :**

2. Panel antigens

3. Recombinant antigens

(-)

4. Environmental Survey  
(qPCR, culture)

(+)

5. "A la carte" antigens from  
the patient's environment

(+)

6. Specific inhalation challenge  
or antigenic eviction

(-)

(+)

