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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

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°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 2m^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

319 **Declaration of interest statement**

320 The authors have no conflicting interests.

321

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506

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>		
Bird breeder	(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	
Wood worker	sawdust, fir, poplar, niagon, iroko, sapeli		
Miller & baker	corn, wheat, oats, rye, barley		
Pets shop worker	Fecal extract of rodents	bat, guinea pig, hamster, ind pig, gerbil, white mouse	
Circumstance	<i>Somatic antigens</i>		
Farmer (panel 1)	<i>Lichtheimia corymbifera</i>	<i>Walleimia sebi</i>	<i>Eurotium amstelodami</i>
	<i>Saccharopolyspora rectivirgula</i>	<i>Thermoactinomyces vulgaris</i>	<i>Saccharomonospora viridis</i>
Farmer (panel 2)	<i>Fusarium solani</i>	<i>Aspergillus versicolor</i>	<i>Aspergillus ochraceus</i>
	<i>Penicillium chrysogenum</i>	<i>Rhodotorula rubra</i>	mesophilic <i>Streptomyces</i>
Housing (panel 1)	<i>A. versicolor</i>	<i>P. chrysogenum</i>	<i>Cladosporium sphaerospermum</i>
	<i>Stachybotrys chartarum</i>	<i>Mucor racemosus</i>	<i>Alternaria alternata</i>
Housing (panel 2)	<i>Schizophylum commune</i>	<i>Trichoderma pseudokoningii</i>	<i>Fusarium oxysporum</i>
	<i>Acremonium strictum</i>	<i>Rhodotorula rubra</i>	<i>Laceyella sacchari</i>
Cheese-maker	<i>Acarus siro</i>	<i>Penicillium camemberti</i>	<i>Penicillium roqueforti</i>
	<i>Mucor spinolosum</i>	<i>Geotrichum candidum</i>	
Pork-butcher	<i>Penicillium nalgiovense</i>	<i>G. candidum</i>	
Jacuzzi	<i>Mycobacterium avium</i>	<i>Mycobacterium phocaicum</i>	
Metalworking fluid	<i>Mycobacterium immunogenum</i>	<i>Bacillus simplex</i>	<i>Pseudomonas oleovorans</i>
		<i>Fusarium solani</i>	
Plaster-maker	<i>S. rectivirgula</i>	<i>T. vulgaris</i>	<i>Aspergillus fumigatus</i>
	<i>Penicillium frequentans</i>	<i>P. chrysogenum</i>	<i>A. versicolor</i>
Sugar cane	<i>L. sacchari</i>	<i>S. viridis</i>	<i>Thermocrisum municipale</i>
	<i>T. vulgaris</i>	<i>A. fumigatus</i>	
Compost	<i>S. rectivirgula</i>	<i>T. vulgaris</i>	<i>S. viridis</i>

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

(-)

(+)

