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Immunological Detection of Wood Decay Fungi—An Overview of Techniques Developed from 1986 to the Present[1,2]

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During the past decade, diagnostic methods for detecting incipient wood decay *in vitro* and *in situ* have been developed using antibodies to target fungal antigens. Antibodies are potentially ideal probes for detecting fungal biodeterioration because they are specific and can quantitate fungal antigens within a complex structure such as wood. Both polyclonal and monoclonal antibodies to various fungal components have been utilized separately and in concert for immunoblotting, enzyme immunoassays, particle agglutination assays, and chromatographic 'dipstick' assays. This paper provides an overview of the challenges encountered and progress made in the field of forest products immunodiagnostics since 1986, Published by Elsevier Science Limited

INTRODUCTION

Techniques for detecting biodeterioration of wood have traditionally included visual and microscopic examination (Wilcox, 1964), culturing of fungi from wood (Nobles, 1965), direct chemical staining (Eslyn, 1979), and sonic and electronic resistance methods (Dunlap, 1981; Shortle, 1982; Ross *et al.*, 1994). Sounding of wood, radiography, and mechanical probing devices are still commonly used to detect advanced decay; however, early stages of decay are difficult to assess by any technique (Highley *et al.*, 1994).

Some techniques are designed to detect viable decay fungi, and others detect residual nonviable fungal metabolizes. All techniques require some specific skill or expertise for interpretation.

Direct staining of core samples with chemical indicators has been somewhat successful in detecting early decay, but results are often ambiguous (Highley *et al.*, 1994). A common technique for differential histochemical staining, the Picro Analine Blue–Safranin staining procedure (Wilcox, 1964), is tedious and time-consuming, and differentiation between the wood cell walls and hyphae is often subjective (Clausen & Ferge, 1995). Culturing directly on agar medium is relatively simple (Nobles, 1965), but antibiotics must be incorporated to suppress bacterial growth. Even then, molds sometimes quickly cover the agar surface, obscuring the growth of wood decay fungi. Standard incubation times of 3 weeks are needed to allow for slow-growing fungi to appear. The Shigometer, an electronic-type detector that measures wood resistance, has been used to detect incipient internal decay in trees and utility poles (Shortle, 1982). This instrument gives unreliable results when the moisture content of the wood is greater than 38% (Morris & Dickenson, 1984).

Wood that has lost only 1% of its weight because of decay will frequently exhibit a 50% loss in strength measured as toughness (Richards, 1954). Proper diagnosis of early decay allows for appropriate remedial treatment with preservatives to arrest decay prior to loss of structural integrity. In the past decade, researchers have successfully developed methods for detecting incipient wood

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decay *in vitro* and *in situ* using antibodies to target fungal antigens. These methods could provide building inspectors, structural engineers, consultants, and consumers with sensitive, rapid, and simple methods for maintaining in-service wood products. This review provides an overview of immunodiagnostic techniques developed in the past decade directed against wood decay fungi.

IMMUNOLOGICAL PROBES

Fungal proteins and polysaccharides are able to elicit an immune response when introduced into a higher animal, typically a rabbit, resulting in antibody production. Antibodies have the capacity to specifically recognize, bind, and neutralize certain foreign antigens (Clausen & Green, 1997). Thus, antibodies are an ideal probe for the detection of fungi that cause biodeterioration because they can be highly specific and quantitatively determine fungal antigen concentration from highly complex structures, such as wood (Palfreyman *et al.,* 1987).

Two types of antibodies, polyclonal and monoclonal, can be produced. General characteristics of both types of antibodies are given in Table 1. Polyclonal antibodies recognize multiple chemical sites, i.e. epitopes, on the antigenic molecule and, in general, are produced *in vivo* by immunization. Monoclonal antibodies recognize one specific epitope or protein sequence on an antigenic molecule (Goding, 1986), and are usually produced *in vitro* by cell culture. Depending on the type and format of the immunoassay, one or both types of antibody may be necessary to achieve a desired result.

Advances in the field of immunodiagnostics have resulted in many applications of this technology to the detection of incipient wood decay. Clausen & Green (1997) described many of these techniques in detail.

Table 1. General Characteristics of Monoclonal and Polyclonal Antibodies (Promega, 1993)

Monoclonals	Polyclonals
Specific for a single epitope	Large population of epitope specificities
Possible low affinity	Multiple, varying affinities
May be difficult to produce desired antibody	Possible cross-reactivity
Time-consuming to produce	Relatively fast to produce
Quantities theoretically unlimited	Limited quantities
Expensive	Inexpensive
Possible problems with detection on Western blots	Usually useful for Western blots
Can usually use impure antigens to produce	May be difficult to produce antibodies from some antigens

Polyclonal antibodies have been generated against a number of brown-rot and stain fungi: *Serpula lacrymans* (Palfreyman *et al.,* 1988; Vigrow *et al.,* 1991), *Postia placenta* (Goodell & Jellison, 1986; Jellison & Goodell, 1988, 1989), *Lentinus lepideus* (Palfreyman *et al.,* 1987, 1988; Clausen *et al.,* 1990; Glancy *et al.,* 1990), *Trametes versicolor* (Palfreyman *et al.,* 1987, 1988), *Ophiostoma piceae* (Luck *et al.,* 1990), and a mixture of antigens from *Coniophora puteana, Postia placenta, Serpula incrassata, Gloeophyllum trabeum,* and *Antrodia carbonica* (Clausen *et al.,* 1991b). Typically, polyclonal antiserum from basidiomycetes cross-reacts strongly with other basidiomycetes, but cross-reactions can be partially or totally eliminated by absorption of the antiserum with the cross-reacting organism (Palfreyman *et al.,* 1988; Glancy *et al.,* 1989; Clausen *et al.,* 1991b; Clausen, 1994a).

Various preparations have been used for polyclonal antibody formation. These include partially purified *Postia placenta* cell walls (Jellison & Goodell, 1988, 1989), whole mycelium from liquid culture, extracellular culture filtrate from *Postia placenta, Trametes versicolor, Lentinus lepideus, Tyromyces palustris, Gloeophyllum trabeum, Serpula incrassata, Antrodia carbonica, Coniophora puteana,* or *Phialophora mutabilis* (Goodell & Jellison, 1986; Goodell *et al.,* 1988; Daniel & Nilsson, 1990; Kim *et al.,* 1991; Clausen *et al.,* 1990), and partially purified hemicellulases extracted from wood decayed by *P. placenta* (Green *et al.,* 1989; Clausen, 1994a).

During monoclonal antibody (mAb) production, crude immunizing antigen may be adequate because clones are selected for recognition of specific antigens from a pool of hybridomas. Fungal enzymes, which are heavily glycosylated and difficult to purify, do not necessarily act like bacterial antigens, which are typically nonglycosylated, during monoclonal antibody production. Jellison & Goodell (1986) first produced monoclonal antibodies to *P. placenta* using extracellular liquid culture filtrates as immunogen. Clausen *et al.,* (1993) reported that glycoprotein immunogens most commonly result in monoclonal antibodies that are specific to the carbohydrate moiety; the xylanase of *P. placenta* has a carbohydrate content of 65%. Only one antixylanase monoclonal antibody of eight proved to be protein-specific after chemical and enzymatic modification of the *P. placenta* xylanase antigen (Clausen *et al.,* 1993).

Glancy and Palfreyman (1993) produced monoclonal antibodies to both whole cell mycelial extracts and extracellular antigen extracts of *Serpula lacrymans.* Cross-reactions were noted between *S. lacrymans* mAbs and enzyme-labeled reagents, resulting in high backgrounds. Most mAbs reacted with surface and strand mycelium and fruiting bodies, but were uniformly nonreactive with spores or decayed wood extracts. Daniel *et al.* (1991) produced mAbs to purified Mn peroxidase for the detection of peroxidase in *Phanerochaete chrysosporium*-infected wood by ELISA and immunolabelling.

IMMUNOFLUORESCENCE

Immunofluorescence was examined by Goodell *et al.* (1988) and Toft (1993) as a means of sensitive, qualitative analysis for detecting basidiomycete fungi in wood. Goodell *et al.* (1988) heat-fixed mycelium from liquid culture to glass slides before labeling with polyclonal antiserum and tagging with anti-rabbit antibody conjugated to fluoresceneisothiocyanate (FITC). Goodell *et al.* (1988) also successfully labeled *P. placenta* decayed wood sections that had been oven-dried or wet-fixed. Toft (1993) labeled 50-µm-thick wood slices infected with *Serpula lacrymans* with anti-*Serpula* antiserum. Wood slices were tagged with a secondary anti-rabbit antibody conjugated to FITC and a tertiary label for an amplified signal. Both groups report nonspecific absorption of antisera to other wood decay basidiomycetes. F. Green (unpublished results) observed fungal hyphae of *P. placenta in situ* using plastic Epon-embedded wood specimens, polyclonal anti-rabbit serum, and goat-anti-rabbit labeled FITC antibody (Fig. 1).

Polyclonal antisera to both the cell wall fraction and extracellular filtrate of a liquid culture of *Postia placenta* bound to the extracellular slime of the fungus (Goodell *et al.*, 1988). They observed no specific fluorescence of hyphae in oven-dried *P.* placenta-infected wood sections. In wet-fixed samples of *P. placenta* decayed wood, immunofluorescence demonstrated hyphae in cross- and longitudinal sections of *Postia*-infected wood, although the resolution was not sufficient to distinguish between fluorescence in hyphal walls and the slime layer. Cross-absorption of this antisera against the nondecay organism *Rhizoctonia solani* did not completely eliminate cross-reactive fluorescence (Goodell *et al.*, 1988). High levels of cross-reactivity with nondecay organisms were also reported with antisera to partially purified glycoprotein or the 'whole fungus'. Toft (1993) found that it was impossible to prevent nonspecific absorption of *Serpula lacrymans* IgG to the fungal mycelia of *S. lacrymans, S. himantioides, C. puteana, Antrodia sinuosa,* and *Paxillus panuoides,* either by fractionation of the immunoglobulins or chemical or enzymatic treatment of the mycelium.

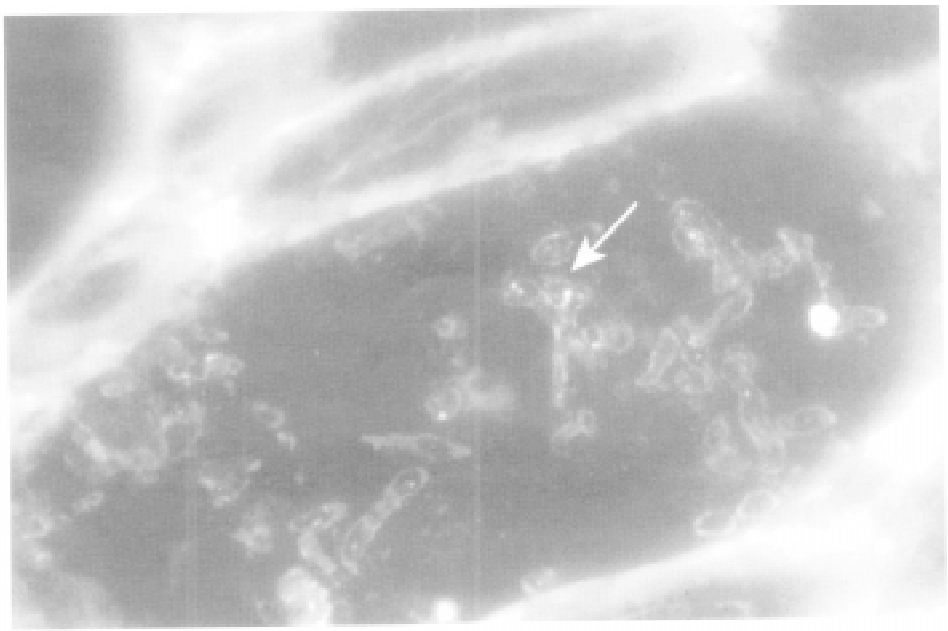

Fig. 1. Immunofluorescence (FITC) of *Postia placenta* in wood. Note the bright staining of fungal hyphae (Green, F., unplublished results).

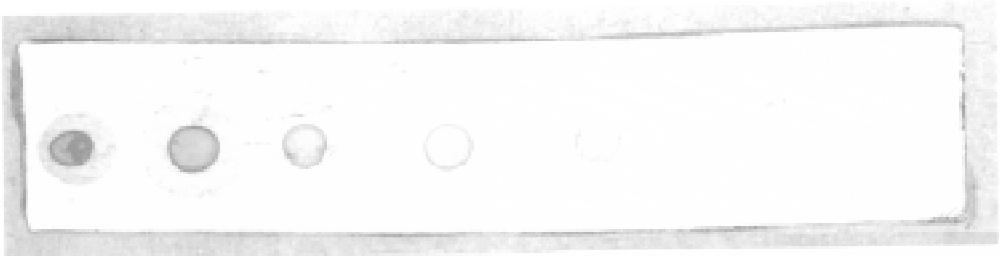

Fig. 2. Immuno-dot blot showing 1:10 dilutions of fungal antigen extracted from decayed wood probed with anti-hemicellulase antibody (Clausen & Green, 1997).

He concluded that it would be impossible to use his anti-*Serpula* antiserum for *in situ* detection of *S. lacrymans.*

IMMUNOBLOTTING

Immunoblotting can also be a sensitive, qualitative method for fungal detection. Antigens bound to nitrocellulose paper are probed with antibody and visualized by an enzyme-substrate reaction. Several variations of this technique have been examined for use as immunodiagnostic tools (Fig. 2). Glancy *et al.* (1989) prepared polyclonal rabbit antibodies to *L. lepideus* with a whole cell antigen preparation. Lime *(Tilia* sp.) sapwood blocks, decayed by *L. lepideus* and exhibiting a variety of weight losses, were ground in phosphate-buffered saline (PBS), and dilutions of the wood extract were screened in the dot-immunobinding assay. Their results indicated that dot-immunoblotting was more sensitive than microbiological culturing for detecting six strains of *L. lepideus* in distribution poles.

Clausen *et al.* (199lb) prepared a multivalent rabbit polyclonal antibody to a sterile culture filtrate of six common brown-rot fungi: *Postia placenta, Gloeophyllum trabeum, Antrodia carbonica, Lentinus lepideus, Serpula incrassata,* and *Coniophora puteana.* This heterogeneous antiserum was able to detect five of the six fungi from infected wood extracts with very weak cross-reactions to the white-rot and nondecay mold and sapstain fungi tested. Clausen *et al.* (1990) recommended that simple dot-immunoblots using polyclonal antiserum could be a useful preliminary test for detecting incipient decay.

Western blot analysis (Towbin *et al.,* 1979) (Fig. 3) is a time-consuming, yet precise means of identifying protein specific and reproducible differences among fungal isolates. Fungal antigens are electrophoretically separated, according to molecular mass on an SDS–polyacrylamide gel. Protein antigens are

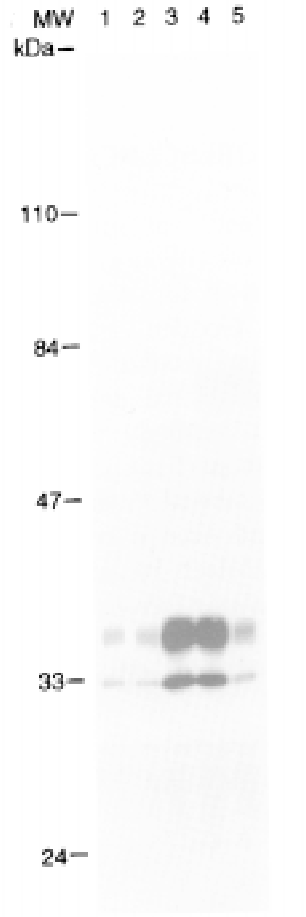

Fig. 3. Western blot analysis after SDS–PAGE of xylanase, indicating binding patterns of the monoclinal antibodies. Molecular weight standards are also indicated (Clausen *et al.,* 1993; Clausen & Green, 1997).

then electrophoretically transferred to nitrocellulose paper and subsequently probed with antisera. This technique may utilize either polyclonal or monoclonal antibodies as probes. Vigrow *et al.* (1991) utilized Western blotting to differentiate isolates of *Serpula lacrymans* from other wood decay basidiomycetes. They found unique antigenic profiles for *Serpula* isolates, even though polyclonal antisera produced to mycelia extracts are generally highly cross-reactive in immunoblots. Glancy *et al.* (1989)

demonstrated alterations in molecular composition of *L. lepideus* and related basidiomycetous fungi using Western blot based on culture age and substrate. The antigenic profile of *G. trabeum* most closely resembled *L. lepideus*, whereas other fungi tested showed marked differences in their antigenic profiles.

AGGLUTINATION ASSAY

Agglutination assays (Fig. 4) are considered to be the most rapid immunodiagnostic procedure (Clausen *et al.,* 1991b). Indeed, they have been utilized in the medical field for more than 50 years. Particulate antigens, i.e. bacteria, may be agglutinated or cross-linked directly by polyclonal antibodies to surface antigens. In the same manner, latex particles coated with antibody will cause visible agglutination in 30s when antigen is present. Interpretation of qualitative results for agglutination assays is subjective; experience is required to evaluate reactions to avoid misinterpretation of weak positives. Latex particles agglutinated at 0% wood weight loss for six brown-rot organisms tested, with no cross-reactivity of a white-rot fungus, mold fungus, or sapstain fungus. Based on the results of Clausen *et al.* (1991b), this assay would be a good presumptive field test for incipient brown-rot decay if performed by qualified personnel.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ELISA assays are performed in 96-well polystyrene plates with a variety of configurations of antigen, antibody, and detection systems. This technique is advantageous because (1) small volumes of reagents are required, (2) multiple tests may be performed in the same plate, (3) readings are automated, and (4) results are quantitative. Indirect ELISA (Fig. 5) is the quantitative immunological method that has been studied most extensively in the field of brown-rot decay. Breuil (1987) and Breuil *et al.* (1990) successfully used enzyme-linked immunosorbent assay for the quantitative detection of the sapstain fungus *Ophiostoma* sp. C28 within infected wood samples using polyclonal antibody. Clausen *et al.* (1990, 1991a,b) and Clausen (1991) also reported positive ELISA results for six brown-rot fungi at 0-13% weight loss in extracts from 50-mg wood samples using polyclonal or monoclinal antibodies prepared against xylanase from *P. placenta*. Daniel & Nilsson (1990) noted strong ELISA reactions in CCA and ammoniacal-copper-treated wood blocks that had been exposed to the soft-rot fungus *Phialophora mutabilis* for 5 months when tested with polyclonal antibody against mycelial extracts from *P. mutabilis.* Cross-reactions with soluble secretory products were likely to be found in soft-rotted wood, i.e. *Trichoderma reesei* cellobiohydrolases and endoglucanases, and to culture filtrate from *Phanerochaete chrysosporium*. Because of the strong ELISA reaction in preservative-treated pine blocks showing insignificant weight loss, Daniels & Nilsson (1990) suggested that ELISA may not give a true impression of the extent of decay. When Kim *et al.* (1989, 1991) compared indirect ELISA with the double antibody sandwich ELISA using anti-*P. placenta* antisera (Fig. 6), the greatest sensitivity was noted in the indirect ELISA. Antisera prepared from the extracellular culture filtrate

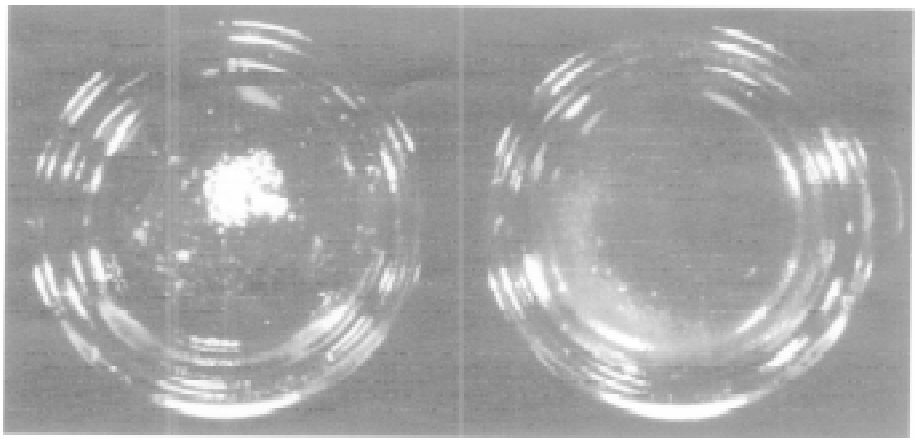

Fig. 4. Agglutination assay: (left) positive control demonstrates visible, grainy aggregates of reagent; (right) negative control shows smooth, homogeneous, non-aggregated latex particles (Clausen et *al.,* 1991b; Clausen & Green, 1997).

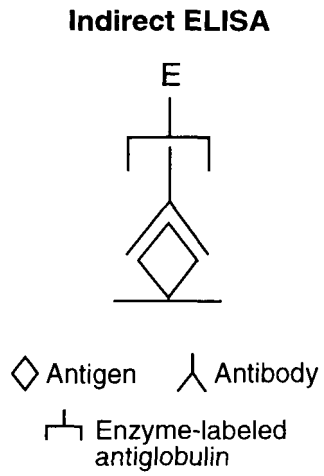

Fig. 5. Schematic of indirect ELISA showing configuration of reagents (adapted from Voller *et al.*, 1979).

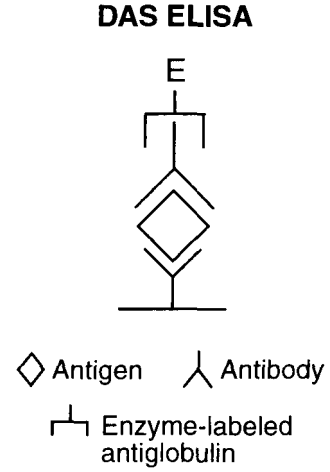

Fig. 6. Schematic of double antibody sandwich (DAS) ELISA showing configuration of reagents (adapted from Voller *et al.*, 1979).

of *Postia placenta* nonspecifically detected white- and brown-rot fungi (Kim *et al.*, 1991). Goodell & Jellison (1986) and Jellison & Goodell (1988) were able to detect a significant ELISA reaction as early as 10 days after inoculation, using wood samples from a standard soil-block assay (ASTM, 1991) and polyclonal rabbit antiserum to *P. placenta*. They found less specificity, but greater sensitivity for the indirect ELISA method, and the opposite was true for the double antibody sandwich ELISA.

MODIFIED ELISA

Breuil *et al.* (1990) first described a modified ELISA that was performed directly on thin sections of wood for the detection of the sap stain fungus, *Ophiostoma* sp. Thin sections of infected wood served as the carrier substrate to give a final aqueous color substrate reaction. Utilizing polyclonal antibodies against whole mycelium of *Ophiostoma* sp., Breuil *et al.* (1990) found that the same amount of fungal antigen was detected in 20-µm-thick wood slices as in wood ground in a Wiley mill to 20 or 60 mesh. This method could be of great benefit for the rapid diagnosis of staining fungi in wood before discoloration is evident.

Clausen (1991) used polyester cloth as a porous adsorbent in a modified ELISA, called a 'cloth ELISA'. The polyester adsorbent acted as an antigen carrier substrate in an otherwise aqueous ELISA reaction conducted in a 96-well polystyrene plate. The sensitivity of the cloth ELISA was similar to the sensitivity for the standard plate ELISA.

DIPSTICK ASSAY

The format of a 'dipstick' assay varies with the individual researcher's design. As the term 'dipstick' implies, all formats provide an easy field test that involves dipping a prepared test strip into a liquid sample, resulting in a visible qualitative reaction. Clausen (1991) tested cotton-tipped swabs as the porous antigen absorbent for a dipstick assay. Swabs were dipped in antigen, blocked with bovine serum albumin, then probed with anti-xylanase monoclonal antibody. Even high concentrations of antibody produced a low signal. Cotton swabs were resistant to blocking, so high backgrounds were also a problem. Other dipstick formats also include a nitrocellulose tip attached to a stick that can be immersed in a series of reagents to give a desired color reaction, i.e. a rapid version of an immunoblot (Dewey *et al.*, 1989; Cahill & Hardham, 1994a,b).

CHROMATOGRAPHIC ASSAY

Numerous formats exist for chromatographic strip assays. They utilize either an enzyme–substrate color reaction on nitrocellulose (as depicted in Fig. 3) or, more commonly, colored latex beads labeled with an antibody. This test combines the speed of an agglutination assay (Fig. 4) with the sensitivity of a double antibody sandwich technique (Fig. 6). Clausen (1994a,b) and Clausen & Green (1994) described a dyed particle capture immunoassay that is superior in sensitivity and

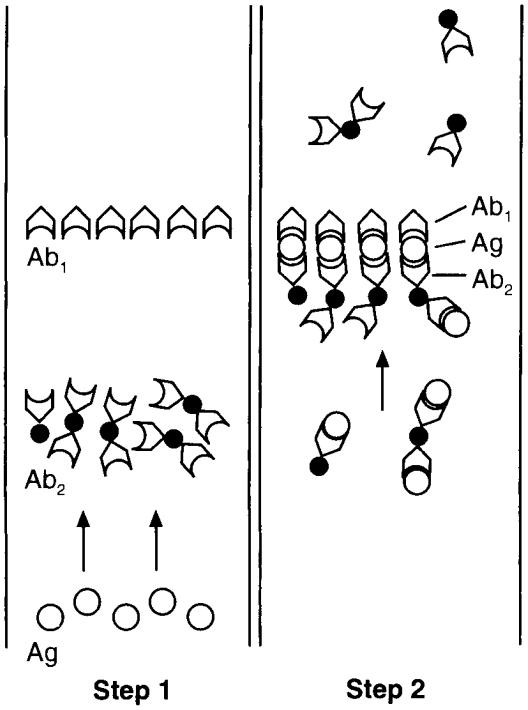

Fig. 7. Schematic of the particle capture immunoassay (PCI), an example of a chromatographic strip assay: step 1, test strip is dipped into fungal extract and extract (antigen) is wicked upward; step 2, fungal antigens captured by Ab_2-labeled beads are sandwiched by a second immobilized antibody (Ab_1) to form colored line indicating a positive result (Clausen & Green, 1994, 1997).

specificity to the indirect ELISA. This assay (Fig. 7) utilizes polyclonal antibody attached to colored latex beads to bind fungal hemicellulases in an antigen preparation. The labeled beads travel through a porous polyester substrate (described previously as a modified cloth ELISA) and become trapped by anti-xylanase monoclonal antibody that is immobilized in another location on the polyester substrate. This technique is specific for brown-rot decay, sensitive to nanograms of xylanase, and is able to detect incipient decay at 0% wood weight loss in a 50-mg wood sample. Current research is examining the mode of extracellular antigen movement through wood structure (Clausen & Ferge, 1995; Clausen, 1996) that enables detection by this method of extracellular enzymes in advance of the fungal hyphae.

OVERVIEW

In general, immunoassays are only as good as the antibodies used to target the fungal antigens. Early in the process, the selection of antigen and type of antibody are critical elements. When designing a broad-based immunoassay to detect brown-rot fungi, a polyclonal antiserum, capable of detecting many species of decay fungi, is often desirable (Kim *et al.,* 1991). This is true only to the extent that the antiserum does not demonstrate nonspecific binding to wood or other groups of nondecay fungi as was noted for polyclonal antisera to whole mycelium or crude extracellular filtrates (Goodell & Jellison, 1986; Palfreyman *et al.,* 1987; Goodell *et al.,* 1988; Daniel & Nilsson, 1991; Kim *et al.,* 1991). Antibodies prepared from heavily glycosylated fungal antigens have a tendency to recognize the nonspecific carbohydrate moieties present in many glycoproteins. Thus, polyclonal antibodies to fungal antigens are only as specific as the antigen preparation. Injecting an animal with whole culture filtrate or mycelium will stimulate an immune response, resulting in antibody production to numerous antigens. As a general rule, there should be no more than 1–2% contaminants in an antigen preparation for the generation of specific polyclonal antibodies (Goding, 1986). Without partial characterization of the antigenic preparation, it will be impossible to assess or characterize the resulting antibody.

Clausen *et al.* (1994) demonstrated that there are antigenic differences between fungal enzymes derived from liquid culture and wood. Varied immunological responses indicate that, ideally, immunizing antigens should originate from a natural substrate, in this case, decayed wood. This is an important consideration when developing immunodiagnostic techniques.

Typically, crude antigen is sufficient for immunization in monoclonal antibody production, because later steps establish precise specificity of clones. However, the glycosylated nature of fungal antigens may necessitate purification of antigen prior to monoclonal antibody production if protein-specific mAb is desired. Wycoff *et al.* (1987) characterized monoclonal antibodies to *Phytophthora megasperma* as carbohydrate-specific or protein-specific. Using chemical or enzymatic modification of the antigen, they were able to determine that most monoclonal antibodies to glycoproteins are carbohydrate-specific. Using partially purified xylanase as antigen, Clausen *et al.* (1993) were able to characterize only one anti-xylanase monoclonal antibody as protein-specific.

Optimizing variables, such as antigen extraction, choice of substrate, or antigen concentration, for an immunoassay is as crucial to the success of the

technique as the antigen purity is to antibody specificity. Breuil *et al.* (1990) and Clausen *et al.* (1990, 1991b, 1994) recognized the importance of non-ionic detergents to improve extraction and solubilization of antigen from the wood. However, Glancy & Palfreyman (pers. comm.) noted interference by the addition of Triton X-100 in their ELISA assay. Gardas & Lewartowska (1988) showed that several detergents, including Triton X-100, prevent binding of protein to plastic surfaces at concentrations equal to or greater than their critical micelle concentration. The target enzyme used by Clausen *et al.* (1990, 1991b, 1993, 1994) was an extracellular polysaccharidase, and the addition of 0.1% Triton X-100 aided the release of the enzyme from hyphal sheath by dissolving the sheath. Any inhibition caused by the solubilizing detergent becomes a trade-off with the increased test sensitivity caused by the release of minute amounts of enzyme from the solubilized sheath. The monoclonal antibodies of Glancy and Palfreyman may have recognized a cellular component that is blocked in the presence of Triton X-100. Some antibodies, particularly monoclonals, recognize epitopes involving secondary or teriary structures that are altered when the antigen is denatured or applied to membranes (Promega, 1993). Targeting a specific antigen (e.g. cell wall, degradative enzyme) and determining which morphological form of the organism contains the specific antigen are important criteria when designing an immunoassay.

Methods for extracting antigen vary, according to the target antigen. During *in situ* immunolabeling experiments with polyclonal antibodies to *Phialophora mutabilis,* Daniel & Nilsson (1991) extracted antigen from decayed wood blocks in carbonate buffer plus Triton X-100, pH 9.6, a typical buffer for coating microtiter plates. Although extraction and simultaneous coating in high pH buffer saves time, Clausen *et al.* (1991b) and Clausen (1994a) and feel that it is preferential to extract in water or phosphate buffer plus Triton X-100, pH 4.0, and subsequently to coat wells at pH 9.6 when the target antigen is an extracellular fungal enzyme.

When adequate antibodies have been produced, the next step is to configure the components of the specific test. It is important to tailor the type of test format to the sensitivity (Table 2), specificity, and speed required. A review by Portsman & Kiessig (1992) covered all parameters of enzyme immunoassay design. They noted that the solid phase considerably influences sensitivity of the assay. It is also important to determine the optimal antigen and antibody concentrations to develop a successful indirect ELISA. This can be accomplished by running a grid pattern of diminishing concentrations. If the optimal antigen concentration is not calculated, unstable polylayers may form. Ironically, the signal may be lower if the antigen concentration is too high than if it is too low, because excess antigen will stack on bound antigen in the initial coating step, bind primary antibody, and then be washed off, exposing unbound antigen coating the bottom of the microtiter plate. Desired reactions may be inhibited by either excess antigen or antibody. This phenomenon is known as the 'prozone' effect.

Nonspecific polyclonal antibody, a term used frequently in the literature, is contradictory. Polyclonal antibodies may bind nonspecifically to reagents, but all antibodies, by definition, are specific for an antigen. Multiple specificity is implied when discussing polyclonal antibody, unless it is known to be a monospecific polyclonal. Monospecificity may be imparted on a polyclonal antibody by (1) using pure antigen, (2) affinity chromatography, or (3) adsorbing with cross-reacting antigen. In the same manner, monoclonal antibodies may be polyspecific if they recognize (1) carbohydrate, (2) repeating epitopes, or (3) common cellular components. Clones are typically selected from a pool of monospecific hybridomas by selecting clones that recognize a known target antigen.

Specificity is compromised when whole mycelial extracts are used as antigen to produce polyclonal

Table 2. Level of Antigen Detection for Various Immunodiagnostic Techniques

Technique	Type of test	Theoretical sensitivity
Indirect ELISA	Quantitative	1–10 μg (Voller & Bidwell, 1986)
DAS ELISA	Quantitative	1 μg (Voller & Bidwell, 1986)
Western blot	Qualitative	1 ng (Promega, 1993)
Agglutination assay	Qualitative	10 pg (Bangs, 1990)
PCI	Qualitative	67 pg (Bangs, 1990)
Immuno-dot blot	Qualitative	100 pg (Promega, 1993)

antibodies (Vigrow *et al.*, 1991). Goodell & Jellison (1986) noted less specificity, but greater sensitivity in indirect ELISA with polyclonal antibody. They also showed that the opposite was true for double antibody sandwich ELISA. In a comparison by Kim *et al.* (1989) of indirect ELISA with double antibody sandwich ELISA, greater sensitivity was detected in indirect ELISA. Clausen (1994a, b) and Clausen & Green (1994) combined the increased specificity of monoclonal antibody and double antibody sandwich ELISA with the increased sensitivity of polyclonal antibody to design a particle capture immunoassay. The polyclonal antibody first detects a broad range of polysaccharides that are then sandwiched by the monoclonal antibody.

Glancy *et al.* (1989) was the first to conduct dot immunoblot assays on nitrocellulose paper with polyclonal antibodies to the brown-rot fungus, *Lentinus lepideus.* This detection method is more sensitive than microbiological culturing. Breuil *et al.* (1990) was the first to apply an indirect ELISA to thin sections of wood for the detection of the sapstain fungus, *Ophiostoma* sp. Novel techniques such as these show potential for use as forest products diagnostics.

Some inhibitory effects of the wood substrate on indirect ELISA were noted by Jellison & Goodell (1989). They demonstrated that an inhibitory effect seen at high fungal concentrations is due, in part, to phenolics. However, they reported that when using a standardized amount of sample, i.e. infected wood, this slight inhibition does not affect the ability of ELISA to detect fungal antigen.

It has been noted that wood decay basidiomycetes lose antigenicity in extracts from wood blocks in late stages of degradation (Glancy *et al.*, 1990; Vigrow *et al.*, 1991) through either autolysis or proteolysis. Although this is interesting to note, it poses no serious drawback to the development of immunological detection methods, because the overall goal of this research is to detect incipient decay. To this end, ELISA has been successful in detecting decay fungi from standardized soil-block assays as early as 10 days after inoculation (Jellison & Goodell, 1988). Clausen *et al.* (1991b) were able to detect decay fungi from standardized soil-block assays at 0–13% wood weight loss using indirect ELISA and anti-hemicellulase antibodies. Although ELISA is a sensitive method for the detection of incipient fungal decay, the particle capture immunoassay is at least 100-fold more sensitive and is able to detect an array of aggressive wood decay basidiomycetes at 0% weight loss (Clausen, 1994a; Clausen *et al.*, 1994).

The field of immunodiagnostics has made great progress toward a sensitive field test for incipient decay. As we work to gain widespread recognition of new methodologies, the future holds promise of methods with even greater sensitivity.

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