



US005952178A

United States Patent [19]
Lapidus et al.

[11] **Patent Number:** **5,952,178**
[45] **Date of Patent:** ***Sep. 14, 1999**

[54] **METHODS FOR DISEASE DIAGNOSIS FROM STOOL SAMPLES**

[75] Inventors: **Stanley N. Lapidus**, Bedford, N.H.; **Anthony P. Shuber**, Milford, Mass.

[73] Assignee: **Exact Laboratories**, Maynard, Mass.

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **09/059,713**

[22] Filed: **Apr. 13, 1998**

Related U.S. Application Data

[63] Continuation-in-part of application No. 08/699,678, Aug. 14, 1996, Pat. No. 5,741,650.

[51] **Int. Cl.⁶** **C12Q 1/68**

[52] **U.S. Cl.** **435/6; 435/7.1; 435/7.2; 435/91.1; 435/91.2**

[58] **Field of Search** **435/6, 7.1, 7.2, 435/91.1, 91.3**

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,101,279	7/1978	Aslam	422/99
4,309,782	1/1982	Paulin	4/661
4,333,734	6/1982	Fleisher	436/66
4,445,235	5/1984	Slover et al.	4/144.2
4,535,058	8/1985	Weinberg et al.	435/6
4,683,195	7/1987	Mullis et al.	435/6
4,705,050	11/1987	Markham	128/749
4,735,905	4/1988	Parker	436/174
4,786,718	11/1988	Weinberg et al.	435/6
4,857,300	8/1989	Maksem	435/40.51
4,871,838	10/1989	Bos et al.	536/27
4,981,783	1/1991	Augenlicht	435/6
4,982,615	1/1991	Sultan et al.	73/864.51
5,087,617	2/1992	Smith	514/44
5,126,239	6/1992	Livak et al.	435/6
5,137,806	8/1992	LeMaistre et al.	435/6
5,149,506	9/1992	Skiba et al.	422/102
5,196,167	3/1993	Guadagno et al.	422/56
5,248,671	9/1993	Smith	514/44
5,272,057	12/1993	Smulson et al.	435/6
5,330,892	7/1994	Vogelstein et al.	435/6
5,331,973	7/1994	Fiedler et al.	128/760
5,348,855	9/1994	Dattagupta et al.	435/6
5,352,775	10/1994	Albertsen et al.	536/23.1
5,362,623	11/1994	Vogelstein et al.	435/6
5,369,004	11/1994	Polymeropoulos et al.	435/6
5,378,602	1/1995	Polymeropoulos et al.	435/6

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

A-11325/95	10/1994	Australia .
0 284 362 A2	9/1988	European Pat. Off. .
0 337 498	10/1989	European Pat. Off. .
0 390 323 A2	10/1990	European Pat. Off. .
0 390 323 A3	10/1990	European Pat. Off. .
0 407 789 A1	1/1991	European Pat. Off. .
0 407 789 B1	1/1991	European Pat. Off. .
0 608 004 A2	7/1994	European Pat. Off. .
0 259 031 B1	11/1994	European Pat. Off. .

WO 92/13103	8/1992	WIPO .
WO 93/20233	10/1993	WIPO .
WO 94/00603	1/1994	WIPO .
WO 94/10575	5/1994	WIPO .
WO 95/07361	3/1995	WIPO .
WO 95/09928	4/1995	WIPO .
WO 95/12606	5/1995	WIPO .
WO 95/13397	5/1995	WIPO .
WO 95/15400	6/1995	WIPO .
WO 95/16792	6/1995	WIPO .
WO 95/18818	7/1995	WIPO .
WO 95/19448	7/1995	WIPO .
WO 95/25813	9/1995	WIPO .
WO 95/31728	11/1995	WIPO .
WO 96/01907	1/1996	WIPO .
WO 96/06951	3/1996	WIPO .
WO 96/08514	3/1996	WIPO .
WO 96/12821	5/1996	WIPO .
WO 96/13611	5/1996	WIPO .

OTHER PUBLICATIONS

Sanger F., S. Nicklen and A.R. Coulson (Dec. 1977) "DNA sequencing with chain-terminating inhibitors" vol. 74, No. 12, *Proc. Natl. Acad. Sci. USA* pp. 5463-5467.

Wallace R.B., et al. (1979) "Hybridization of synthetic oligodeoxyribonucleotides to $\Phi\chi$ 174 DNA: the effect of single base pair mismatch" vol. 6, No. 11 *Nucleic Acids Research* pp. 3543-3557.

Coll P., K. Phillips, and F. C. Tenover (Oct. 1989) "Evaluation of a Rapid Method of Extracting DNA from Stool Samples for Use in Hybridization Assays" vol. 27, No. 10 *Journal of Clinical Microbiology* pp. 2245-2248.

Jessup J. M. and G. E. Gallick (Sep./Oct. 1992) "The Biology of Colorectal Carcinoma" *Current Problems in Cancer* pp. 263-328.

Litja A., L. Liukkonen and H. Siitari (1992) "Simultaneous detection of two cystic fibrosis alleles using dual-label time-resolved fluorometry" 6 *Molecular and Cellular Probes* pp. 505-512.

Young G. P., and B. H. Demediuk (1992) "The genetics, epidemiology, and early detection of gastrointestinal cancers" 4 *Current Opinion in Oncology* pp. 728-735.

(List continued on next page.)

Primary Examiner—Nancy Degen

Assistant Examiner—Irem Yucel

Attorney, Agent, or Firm—Testa, Hurwitz, & Thibeault

[57] **ABSTRACT**

The present invention provides methods for preparing a stool sample in order to screen for the presence of indicators of a disease, for example a subpopulation of cancerous or precancerous cells. The methods take advantage of the recognition that cellular debris from cancerous and precancerous cells is deposited onto only a longitudinal stripe of stool as the stool is forming in the colon. Accordingly, methods of the invention comprise obtaining a representative sample, such as a circumferential or cross-sectional sample of stool in order to ensure that any disease indicator, such as cellular debris that is shed by colonic cells, is obtained in the sample.

18 Claims, 4 Drawing Sheets

U.S. PATENT DOCUMENTS

5,380,645	1/1995	Vogelstein	435/6
5,380,647	1/1995	Bahar	435/7,23
5,382,510	1/1995	Levine et al.	435/6
5,409,586	4/1995	Kamahori et al.	204/182.8
5,458,761	10/1995	Kamahori et al.	204/299
5,463,782	11/1995	Carlson et al.	4/661
5,466,576	11/1995	Schulz et al.	435/6
5,468,610	11/1995	Polymeropoulos et al.	435/6
5,468,613	11/1995	Erlich et al.	435/6
5,489,508	2/1996	West et al.	435/6
5,492,808	2/1996	de la Chapelle et al.	435/6
5,496,470	3/1996	Lenhart	210/222
5,508,164	4/1996	Kausch et al.	435/6
5,512,441	4/1996	Ronal	435/6
5,514,547	5/1996	Balazs et al.	435/6
5,527,676	6/1996	Vogelstein et al.	435/6
5,532,058	7/1996	Vogelstein	435/371

OTHER PUBLICATIONS

- Hoss M., et al. (Sep. 17, 1992) "Excrement analysis by PCR" *Scientific Correspondence* pp. 199.
- Sidransky, et al. (Apr. 3, 1992) "Identification of ras Oncogene Mutations in the Stool of Patients with Curable Colorectal Tumors" vol. 256 *Science* pp. 102–105.
- Takeda S., S. Ichii, and Y. Nakamura (1993) "Detection of K-ras Mutation in Sputum by Mutant–Allele–Specific Amplification (MASA)" 2 *Human Mutation* pp. 112–117.
- Leong P. K., et al. (1993) "Detection of MYCN Gene Amplification and Deletions of Chromosome 1p in Neuroblastoma by In Situ Hybridization Using Routine Histologic Sections" vol. 69, No. 1 *Laboratory Investigations* pp. 43–50.
- Thibodeau S.N., G. Bren, D. Schaid (May 7, 1993) "Microsatellite Instability in Cancer of the Proximal Colon" vol. 260 *Science* pp. 816–819.
- Naber S.P.(Dec. 1, 1994) "Molecular Pathology—Detection of Neoplasia" 331 *New England Journal of Medicine* pp. 1508–1510.
- Cave H., et al. (1994) "Reliability of PCR Directly from Stool Samples: Usefulness of an Internal Standard" vol. 16, No. 5 *BioTechniques* pp. 809–810.
- Caldas C., et al (Jul. 1, 1994) "Detection of K-ras Mutations in the Stool of Patients with Pancreatic Adenocarcinoma and Pancreatic Ductal Hyperplasia" 54 *Cancer Research* pp. 3568–3573.
- Charlesworth B., P. Sniegowski and W. Stephan (Sep. 15, 1994) "The evolutionary dynamics of repetitive DNA in eukaryotes" vol. 371 *Nature* pp. 215–220.
- Fearon E. R.(1995) "16 Molecular Abnormalities in Colon and Rectal Cancer" *The Molecular Basis of Cancer* pp. 340–357.
- Ravelingien N., J. C. Pector & T. Velu (1995) "Contribution of molecular oncology in the detection of colorectal carcinomas" 58 *Acta Gastro-Enterologica Belgica* pp. 270–273.
- Duffy M.J.(1995) "Can Molecular Markers Now Be Used for Early Diagnosis of Malignancy?" 41/10 *Clin. Chem.* pp. 1410–1413.
- Blum H.E.(1995) "Colorectal Cancer: Future Population Screening for Early Colorectal Cancer" vol. 31A *European Journal of Cancer*, pp. 1369–1372.
- Ridanpaa M., S. Anttila and K. Husgafvel-Pursiainen (1995) "Detection of Loss of Heterozygosity in the p53 Tumor Suppressor Gene Using a PCR-based Assay" 191 *Path. Res. Pract.* pp. 399–402.
- Smith–Ravin J., J. England, I.C. Talbot, W. Bodmer (1995) "Detection of c-Ki-ras mutations in faecal samples from sporadic colorectal cancer patients" 36 *Gut* pp. 81–86.
- Orlow I., et al. (Oct. 18, 1995) "Deletion of the p16 and p15 Genes in Human Bladder Tumors" vol. 87, No. 20 *Journal of the National Cancer Institute* pp. 1524–1529.
- Hasegawa, Y., et al., (1995) "Detection of K-ras mutations in DNAs isolated from feces of patients with colorectal tumors by mutant–allele–specific amplifications (MASA)" 10 *Oncogene* pp. 1441–1445.
- Loktionov A. and I. K. O'Neill (1995) "Early detection of cancer–associated gene alterations in DNA isolated from rat feces during intestinal tumor induction with 1,2-dimethylhydrazine" 6 *International Journal of Oncology* pp. 437–445.
- Honchel R., K. C. Halling and S. N. Thibodeau (1995) "Genomic instability in neoplasia" vol. 6 *Seminars in Cell Biology* pp. 45–52.
- Deuter R., S. Pietsch, S. Hertel and O. Muller (1995) "A method for preparation of fecal DNA suitable for PCR" vol. 23, No. 18 *Nucleic Acids Research* pp. 3800–3801.
- Dib C., et al. (Mar. 14, 1996) "A comprehensive genetic map of the human genome based on 5,264 microsatellites" vol. 380 *Nature* pp. 152–154.
- Cunningham C. and M.G. Dunlop (1996) "Molecular genetic basis of colorectal cancer susceptibility" 83 *British Journal of Surgery* pp. 321–329.
- Mao L., et al. (Feb. 2, 1996) "Molecular Detection of Primary Bladder Cancer by Microsatellite Analysis" vol. 271 *Science* pp. 659–662.
- Villa E., et al. (May 1996) "Identification of Subjects at Risk for Colorectal Carcinoma Through a Test Based on K-ras Determination in the Stool" vol. 110, No. 5 *Gastroenterology* pp. 1346–1353.
- Nollau P., C. Moser, G. Weinland, and C. Wagener (1996) "Detection of K-ras Mutations in Stools of Patients with Colorectal Cancer by Mutant–enriched PCR" 66 *Int. J. Cancer* pp. 332–336.
- Eguchi S., N. Kohara, K. Komuta, and T. Kanematsu (Apr. 15, 1996) "Mutations of the p53 Gene in the Stool of Patients with Resectable Colorectal Cancer" vol. 77, No. 8 *Cancer Supplement* pp. 1707–1710.
- Nollau P., C. Moser, and C. Wagener (May 1996) "Isolation of DNA from Stool and Bodily Fluids for PCR Amplification" vol. 20, No. 5 *BioTechniques* pp. 784–788.
- Rhyu M. S. (Mar. 6, 1996) "Molecular Mechanisms Underlying Hereditary Nonpolyposis Colorectal Carcinoma" vol. 88, No. 5 *Journal of the National Cancer Institute* pp. 240–251.
- Gyllensten U. B., Allen M. (1995) "Sequencing of In Vitro Amplified DNA" In *Recombinant DNA Methodology II* (Wu, ed) pp. 565–578.

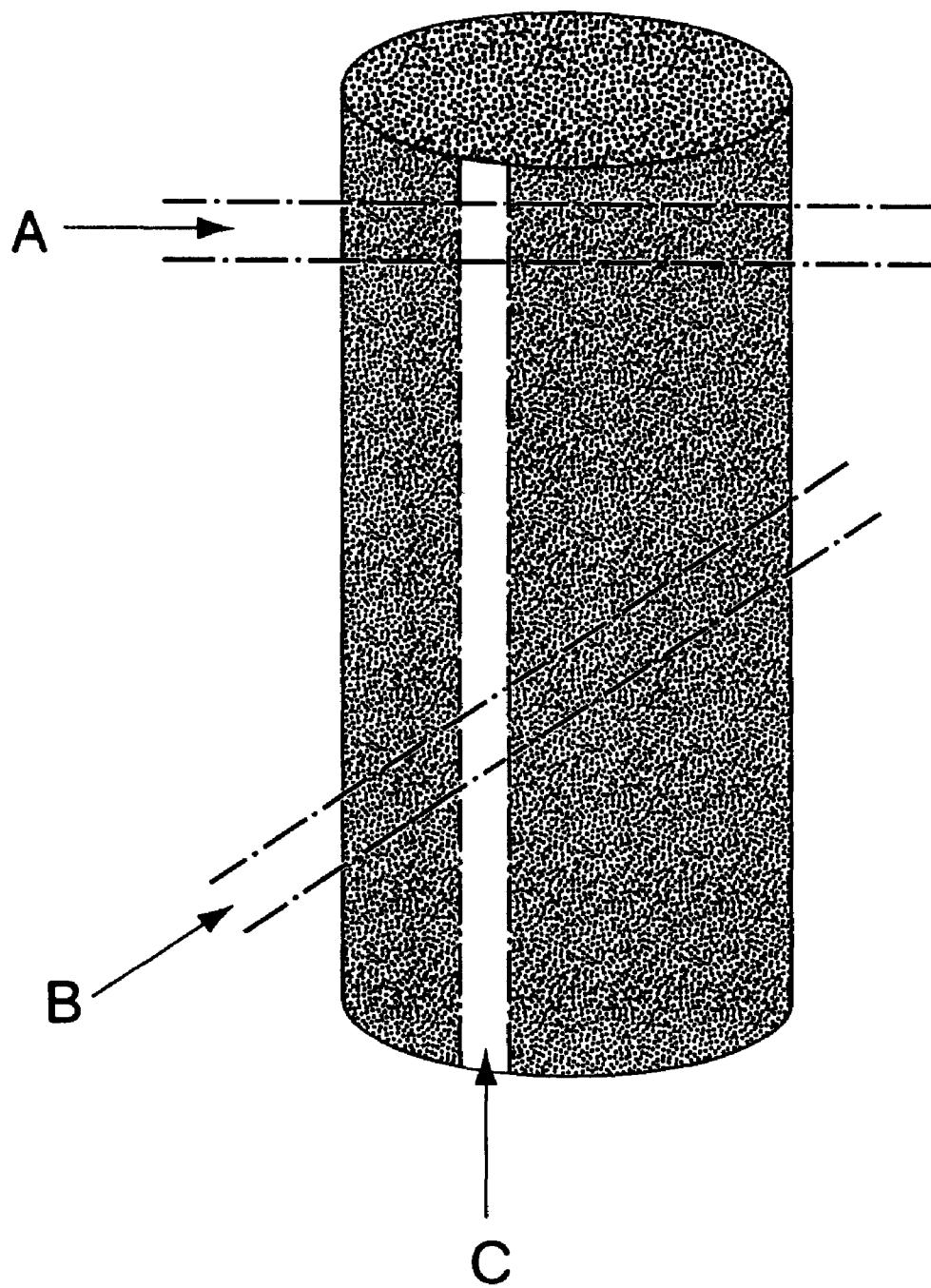


Fig. 1

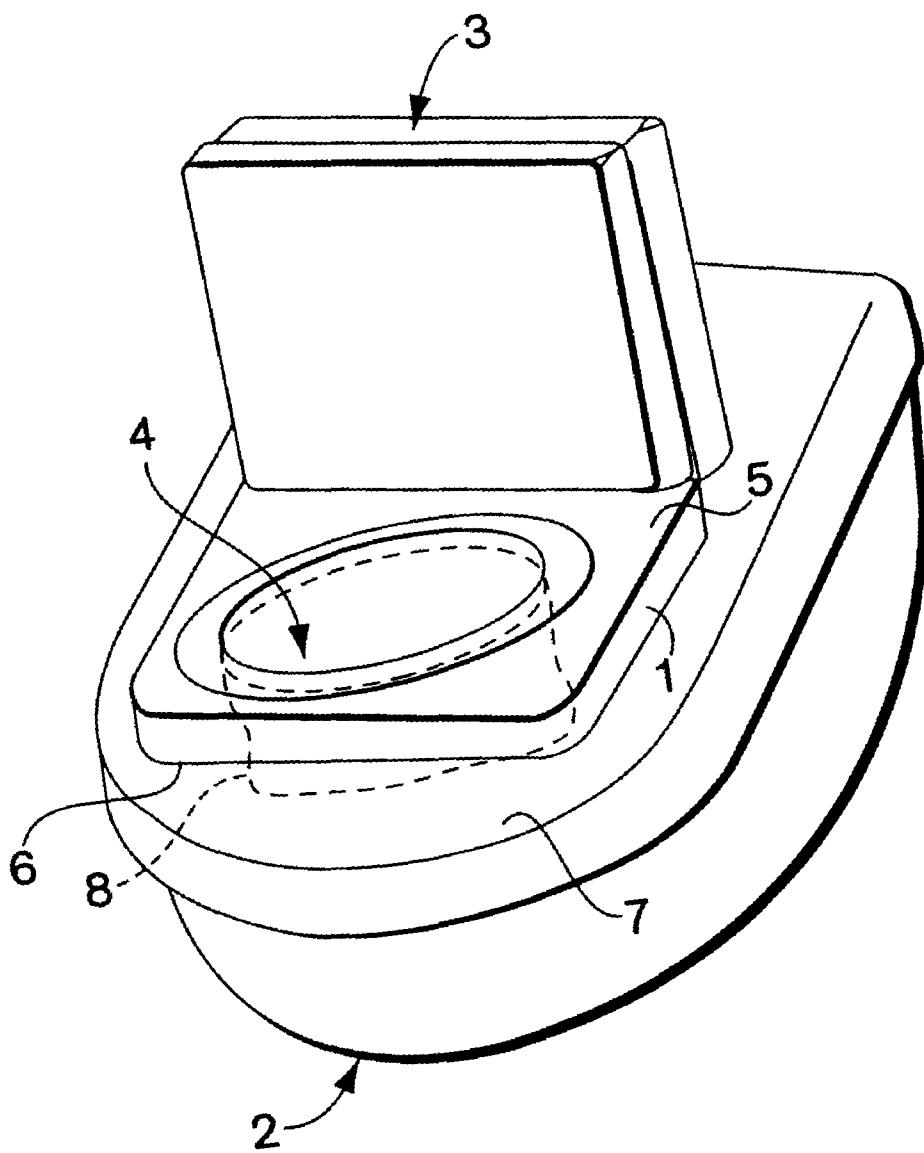


Fig. 2

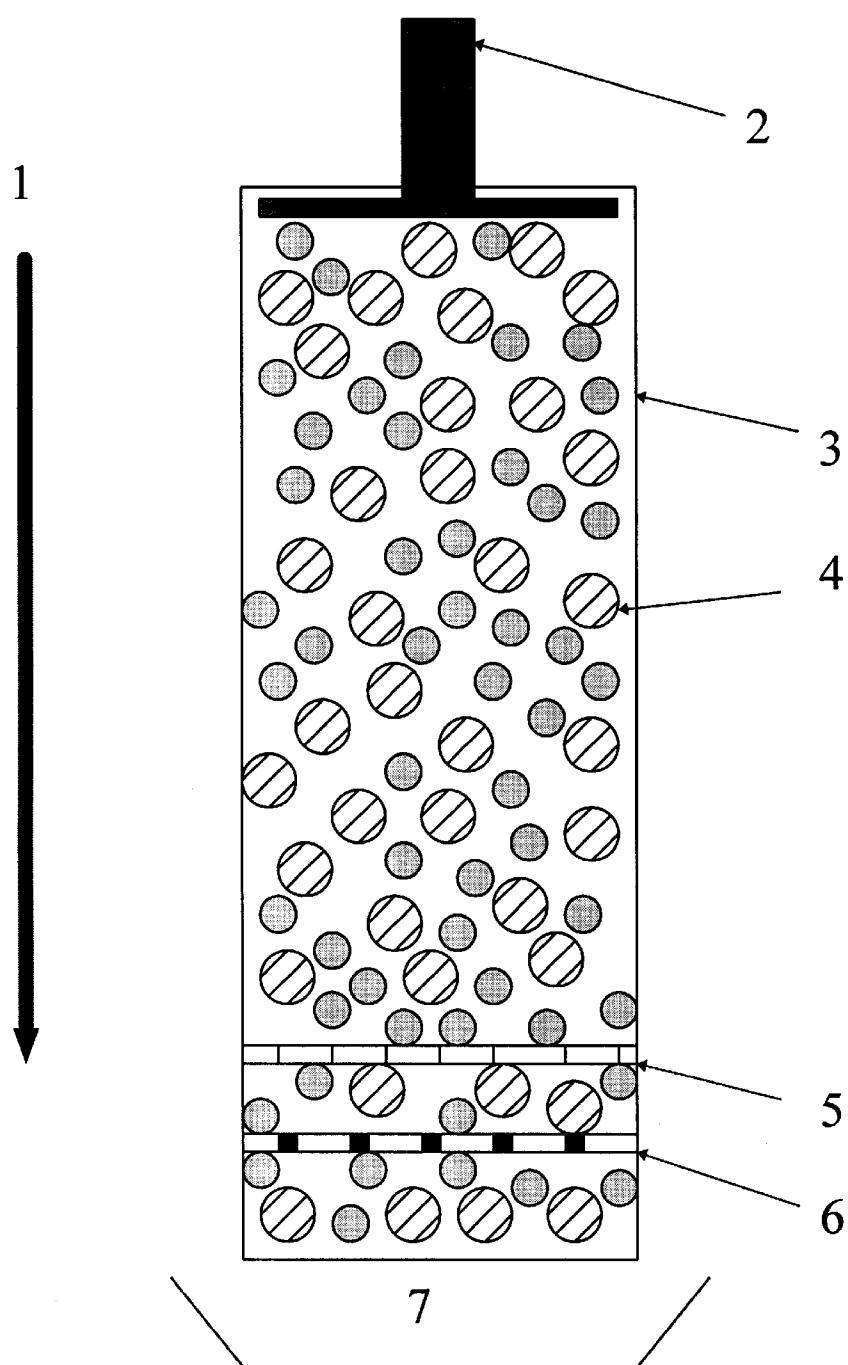


FIG. 3

Explore Litigation Insights



Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.