Oligoclonal bands in multiple sclerosis

Freedman & colleagues (2005) published a consensus statement dealing with CSF analysis for the diagnosis of MS. They recommended that detection of oligoclonal bands (OCB) in the CSF of patients suspected of multiple sclerosis is a "gold standard" which has excellent sensitivity (>95%) and specificity. This section describes in details some fact of OCBs with sample requirements, methodology and examples of the main patterns seen with interpretation are emphasised.

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Facts about oligoclonal bands

- Two or more oligoclonal bands (OCB) in the CSF suggests intrathecal IgG synthesis.
- OCBs are indicative of immune response in the CNS and diagnosis of multiple sclerosis (MS) in patients with clinical suspicion.
- There is no correlation between OCBs in CSF and demyelinating process
- OCB can be present even when the CSF IgG level is normal
- An established oligoclonal response may exist throughout life (except in CNS infections)
- OCB also found in other cases thus OCBs in isolation are meaningless.
- Isoelectric focusing is most sensitive method for detection of the OCB.
- Patterns 1-5, described in this section, are typically common (but real examples). The important patterns in MS are those that demonstrate "intrathecal IgG synthesis" (pattern 2 and 3).

Sample requirement

For the detection of oligoclonal bands, paired serum and CSF samples are required. Blood must be taken at the same time as the lumbar puncture (LP), if not then within two to three weeks of the LP. Results cannot be relied upon beyond this time limit (half life of serum IgG is about 23 days).

This example demonstrates the effect of unpaired samples on oligoclonal bands. Serum was taken six weeks after the LP and contains extra bands which are not reflected in the CSF. The absence of bands in the serum corresponding to those present in the CSF might lead to incorrect interpretation of intrathecal IgG synthesis (pattern 2 and/or 3). Due to the time delay the relvant IgG may have been cleared from the serum. Consequently, these results could not be relied upon without another LP.

Isoelectric focusing method

The most sensitive method for the detection of oligoclonal bands (OCB) is isoelectric focusing (IEF). The principle involves the separation of proteins (IgG's) in the paired serum and cerebrospinal fluid (CSF) using agarose gel electrophoresis followed by passive transfer onto nitrocellulose membrane. The separated IgGs are then detected directly by horseradish peroxidase labelled anti-human antibody.

SUMMARY:

GEL SOLUTION: 0.3g agarose, 3.6g sorbitol, 27ml of 10% glycerol and microwave to dissolve.

CASTING GEL: AT 65°C add 2ml (pH 3-10) & 0.5ml (pH 8-10.5) pharmalyte to molten agarose (65-70°C), mix and cast at 65°C.

HYSTERESIS: Keep the casted gel at 4°C in a moist chamber for at least 30 min.

SAMPLE PREPARATION: Serum diluted at 1:400 & 5µl loaded. Vol. (µl) of CSF loaded = (2.5/total protein).

RUNNING THE GEL: Blot the gel with nitrocellulose membrane (NCM). Position the electrode wicks at 7cm apart (1M NaOH (-ve), 0.05M H_2SO_4 (+ve)) on the gel and then the application strip 2cm from +ve electrode. Remove application strip after 20mins. The gel is electrophoresed at 1250 volt/hour.

BLOTTING: Pre-blot gel for 10 sec with NCM and discard it. Blot with another NCM piece for 30min.

BLOCKING: Dry the blot. Incubate in 2% marvel/saline for 30minutes and quick rinse in tap water.

PRIMARY ANTIBODY: Anti-human IgG (gamma chain) peroxidase, ~1:200 dilution in 0.2% marvel/saline and incubate for 60mins.

WASH/TAP WATER/SALINE: Rinses in tap water followed by 5 min in saline.

DEVELOPMENT OF THE BLOT: To 50mL of 50mM sodium acetate (pH 5.1), add 20mg tablet of A.E.C. dissolved in 2.5ml D.M.F/methanol and 30µl of H_2O_2, (20-30mins).

WASH/TAP WATER: Rinse in running tap water, dry and store in dark.

For Interpretation of oligoclonal bands, please refer to examples of most commonly recognised patterns on this site.

Pattern 1: No OCBs seen

No oligoclonal bands in CSF or Serum. **No intrathecal IgG synthesis.**

KEY: S = Serum, C = CSF
The blot number also represents both the pattern and the anode side.

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Pattern 2: OCBs in CSF only

Oligoclonal bands present in CSF only. **Intrathecal IgG synthesis as seen in MS.**

KEY: S = Serum, C = CSF
The blot number also represents both the pattern and the anode side.

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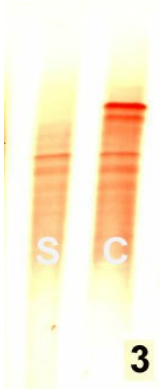
Pattern 3: Identical OCBs in both with extra in CSF



Identical bands in both serum and CSF with extra bands in CSF. **Demonstrates both intrathecal and systemic IgG synthesis. This is also seen in MS.**

KEY: S = Serum, C = CSF
The blot number also represents both the pattern and the anode side.

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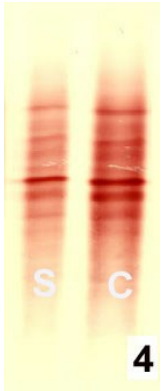
Pattern 4: Identical OCBs in both - mirror



Bands in serum mirror those in CSF. **This suggests systemic IgG synthesis.**

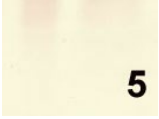
KEY: S = Serum, C = CSF
The blot number also represents both the pattern and the anode side.

(/Images/College-MDS-only/facilities/cis/neuroimmunology/NU6(1).jpg)

Pattern 5: Ladder OCBs

These abnormal "ladder" type identical bands seen both in the CSF and serum are usually **monoclonal** proteins, **suggesting peripheral IgG synthesis.**

KEY: S = Serum, C = CSF
The blot number also represents both the pattern and the anode side.



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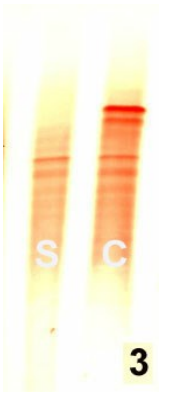
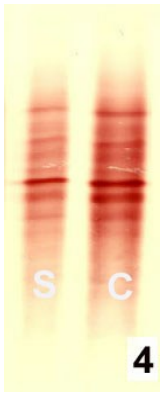
OCBs in other conditions

Conditions	Pattern 2	Pattern 3	Pattern 4
.			
Infection	Yes (CNS)	Yes	Yes (systemic)
Inflammation	Yes (CNS)	Yes	Yes (systemic)
Paraneoplastic	Yes	Yes	Yes
Neoplastic	Rare	Rare	Rare
GBS	No	No	No
Other neuropathies	No	No	Yes
Multiple Sclerosis	Yes	Yes	No
Vascular disease	No	No	Rare
Degenerative disease	No	No	Rare

GBS = Guillain-Barré syndrome, S = serum, C = CSF